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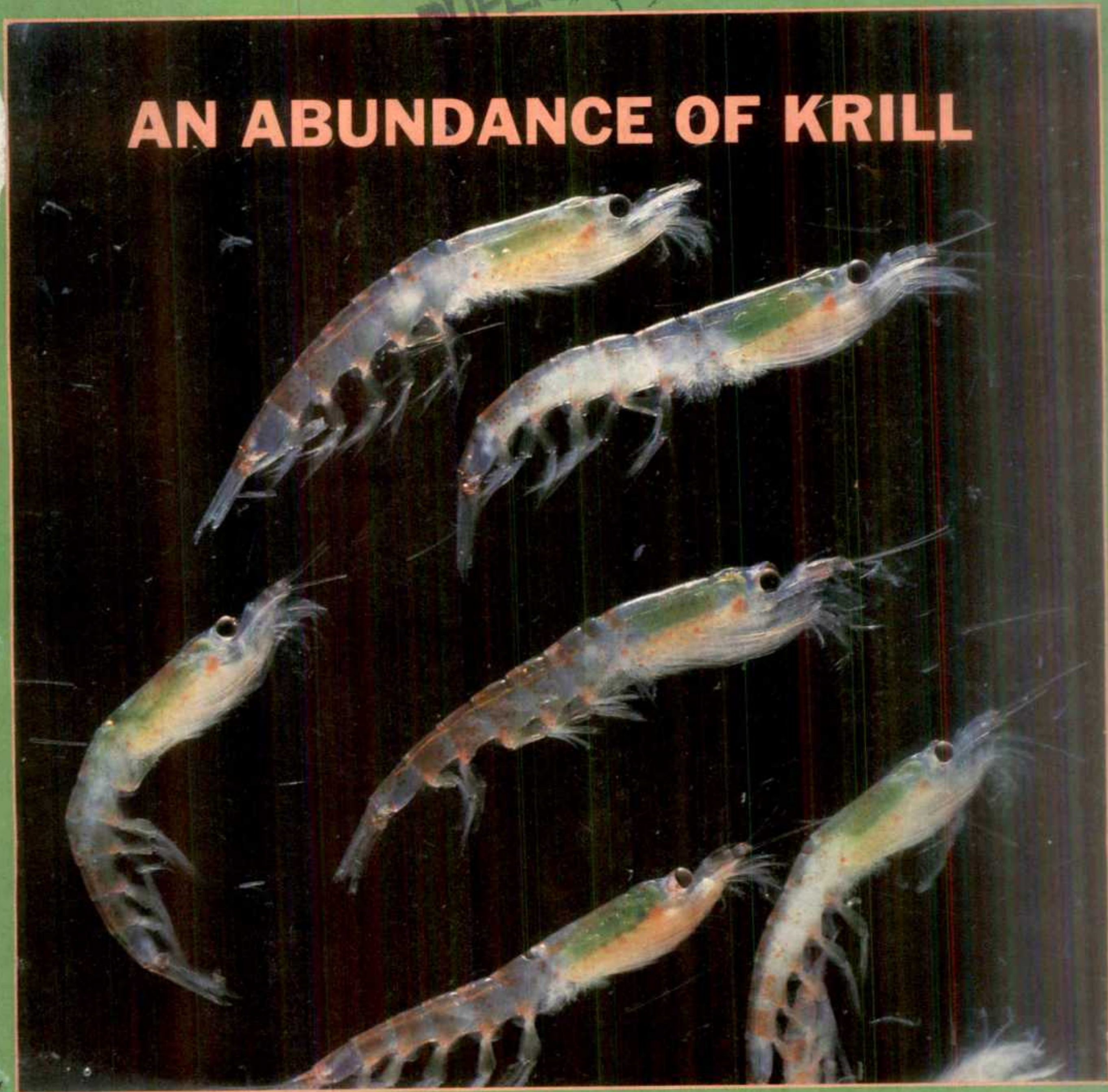

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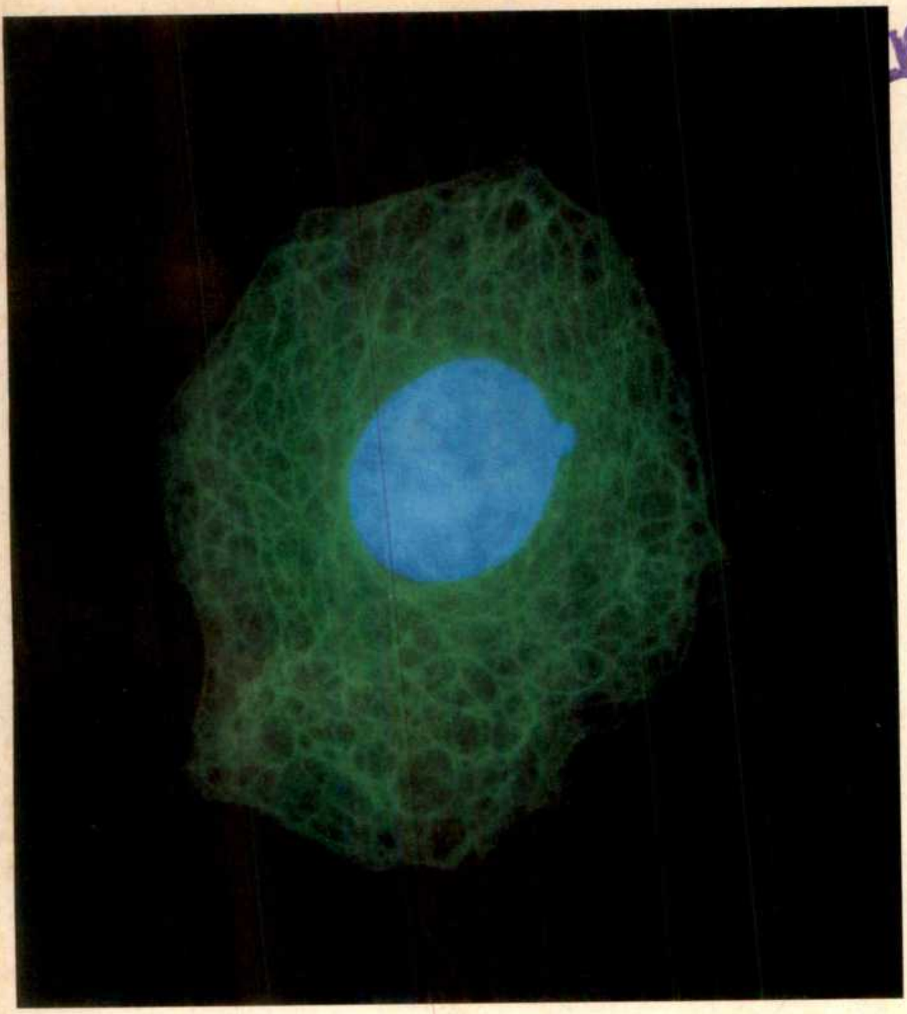

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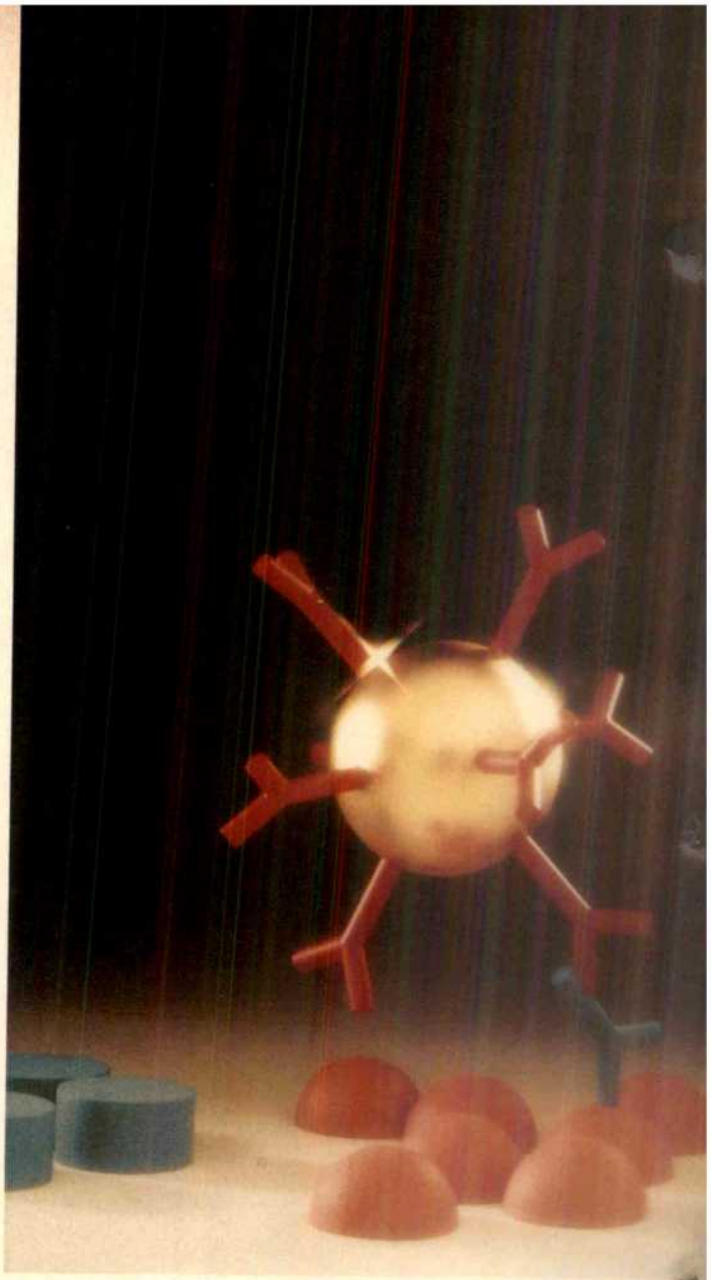

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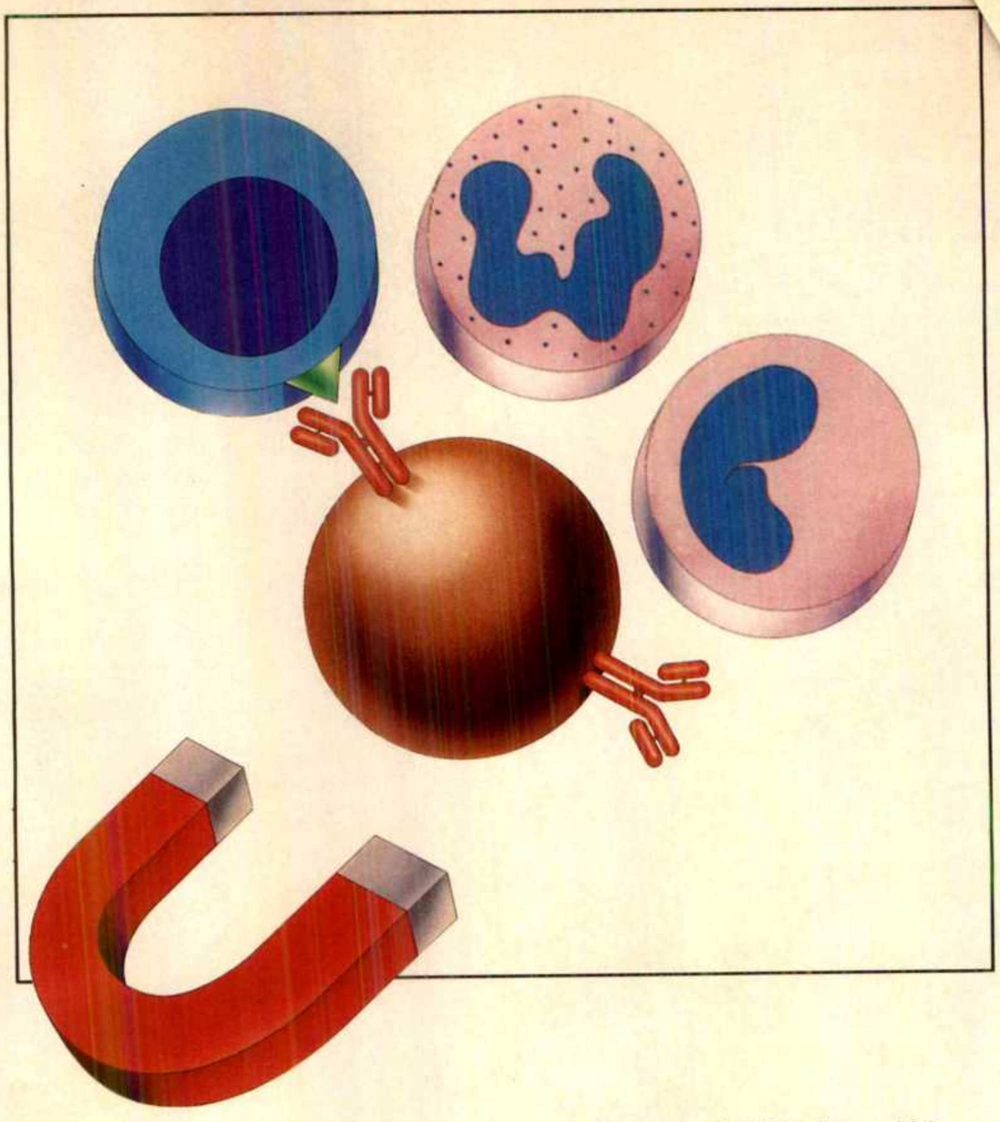

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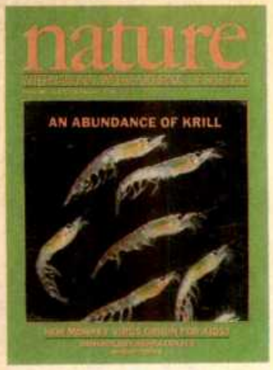

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◀ Present estimates of the abundance of Antarctic krill, dominant component of the diets of many marine animals and important to commercial fisheries, may be grossly inaccurate. See page 338. Cover: Antarctic krill (*Euphasia superba*) near South Georgia. (Inigo Everson.)

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

AIDS-like virus

New clues about the origin of HIV-1 in humans should emerge from the discovery of a chimpanzee lentivirus whose sequence is closely related to that of HIV-1, yet is outside the range of variation of individual HIV-1 isolates. Pages 356 and 288.

Bar to progress

The best properties of high-temperature oxide superconductors have so far been observed only in thin films and tiny grains. Any practical applications must await manufacturing techniques capable of producing high critical current densities in bulk materials. A process capable of making a 5-cm-long bar of 123 superconductor with good current-carrying properties is a step in the right direction (pages 326 and 292). The search for new superconducting compounds continues, and one such (Li_xNbO_2) is described on page 324, featuring a layered structure like the copper oxide compounds, but without a high transition temperature.

Solar-cell 'best'

Recent improvements in the performance of photoelectrochemical solar cells have come from modifications of the semiconductor electrode. Licht and Paramunage have turned their attention to optimizing the cell's other main component, the electrolyte, and claim a best-so-far 16.4% efficiency. Pages 293 and 330.

Muscular dystrophy link

Dystrophin, the protein whose absence in muscle tissue is associated with Duchenne muscular dystrophy, is linked to the muscle membrane through its tight association with glycoproteins. One of these proteins is greatly reduced in Duchenne patients and *mdx* mice, suggesting that its loss may contribute to the pathogenesis of muscular dystrophy. Page 315.

T cells in MS

T cells from the brains of multiple sclerosis patients show only a limited number of receptor gene arrangements, which may offer a new approach to elucidation of the pathogenesis of multiple sclerosis and possibly its therapy. Page 344.

The Sun never sets . . .

During the past decade, round-the-clock measurement of solar spectra has become possible, providing evidence of acoustic oscillations which correlate with changes in solar activity. Factors that might be responsible for such oscillations include changes in solar dimensions, the speed of sound in the Sun, and the magnetic field. Page 322.

Timely technique

Unlike conventional X-ray crystallography the Laue method allows time-resolved structure determination. This has now been put to use in determining for the first time the structure of the short-lived active form of the p21Ras oncoprotein with bound GTP and showing the conformation changes that follow GTP hydrolysis. Pages 309 and 294.

'Primates' reappraised

Two reports of new fossil evidence suggest that many if not all of the so-called archaic primates from the early Cenozoic are not primates at all, and that their closest living relatives are the flying lemurs or cologus. Pages 340, 342 and 291.

Life in carbon cycle

The amount of carbon dioxide in the atmosphere is steadily increasing, but the yearly increments vary around a mean trend. The occurrence of El Niño climatic events is one factor behind this fluctuation, and evidence that biological rather than oceanic processes are involved is discussed in News and Views, page 295.

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1. Nielson, K. and Mathur, E.J. (1990) Strategies 3:17-19.
2. Nielson, K. and Mathur, E.J. Manuscript in preparation.
3. Nielson, K. and Mathur E.J. (1989) U.S. patents filed.
4. Mullis, K.B., and Faloona, F.A. (1987) Meth. Enzymol. 155:335-350.

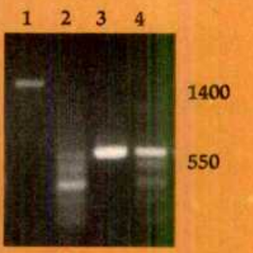

Figure Legend: A photograph of a 1% agarose gel stained with ethidium bromide representing reaction products from PCR amplifications using the GeneAmp™ Kit† from Perkin-Elmer Cetus according to manufacturer's instructions. The reactions were conducted with (lanes 1 and 3) and without (lanes 2 and 4) the inclusion of 1 unit Perfect Match polymerase enhancer. Lanes 1 and 2 represent 100 ng of human genomic DNA amplified with two 26-mer primers separated by 1400 nucleotides. Lanes 3 and 4 represent 100 ng of mouse genomic DNA amplified with two 23-mer primers separated by 550 nucleotides.

Figure 1 shows two examples of *in vitro* amplification reactions that are significantly enhanced by the addition of Perfect Match polymerase enhancer to the polymerase preparation. Note that in lanes 1 and 2, the desired PCR product cannot be detected unless Perfect Match polymerase enhancer is added to the amplification reaction.

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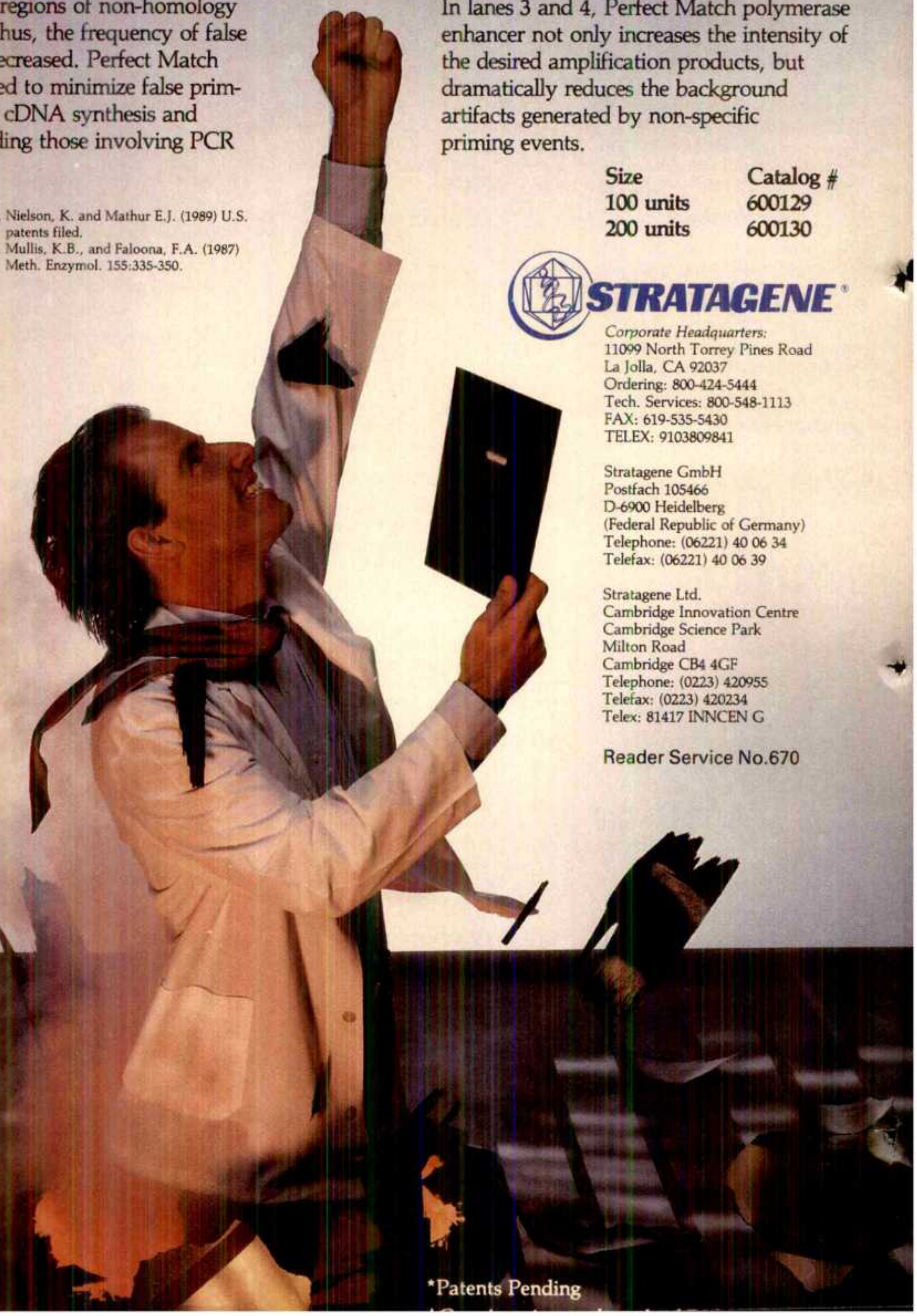

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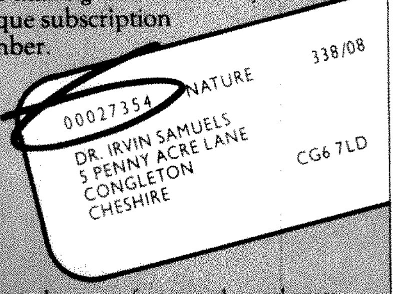

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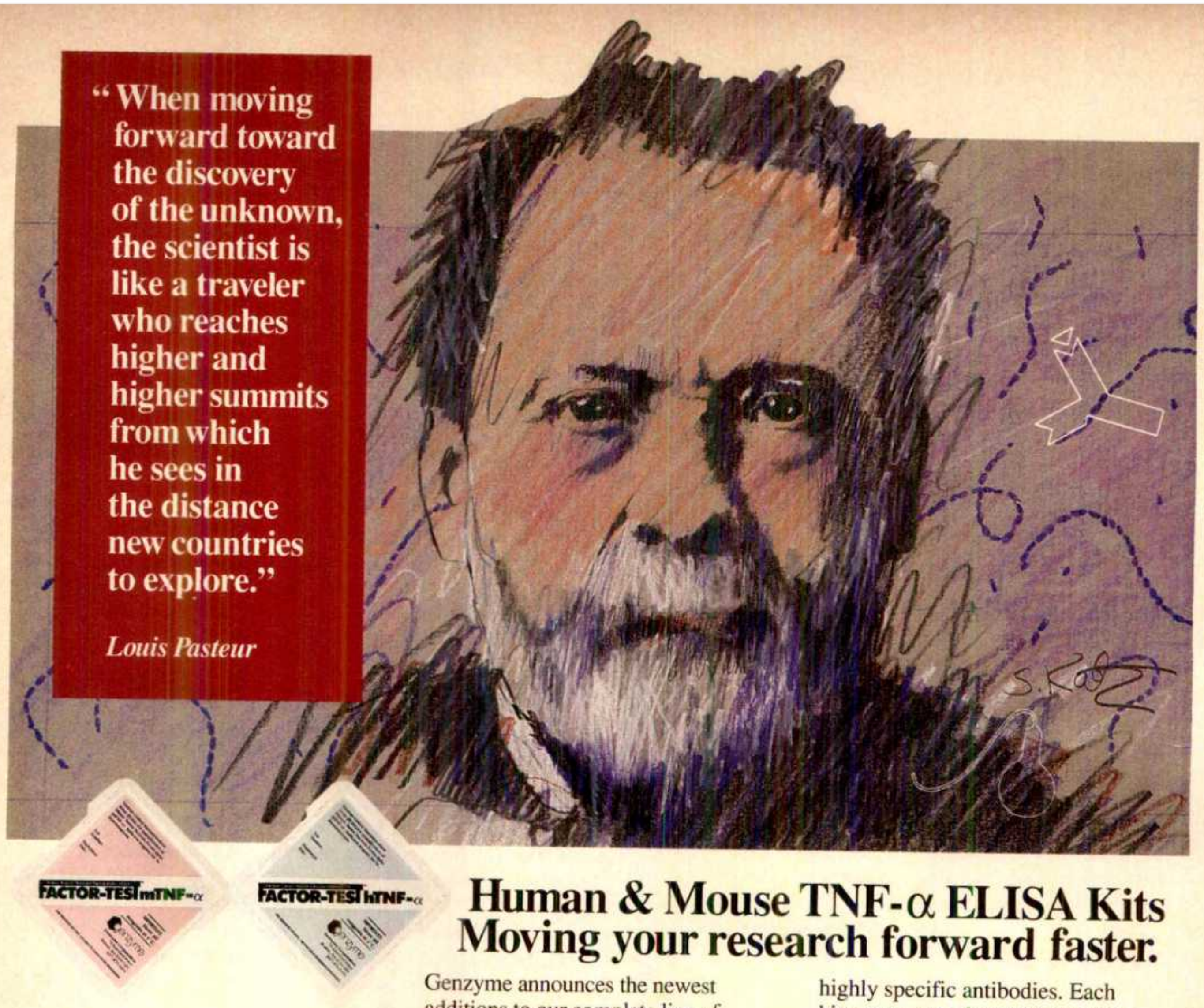

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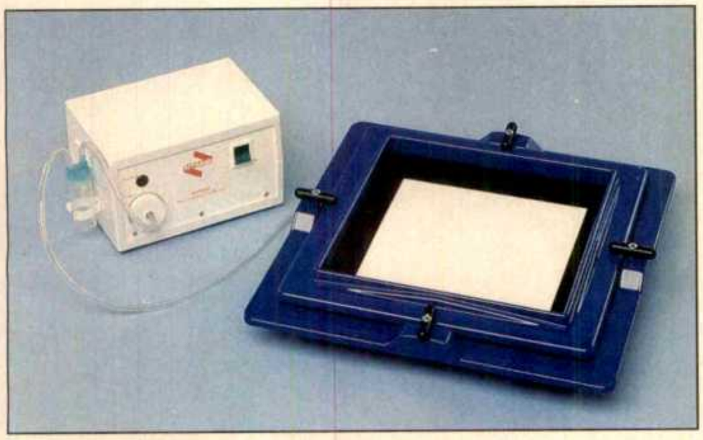

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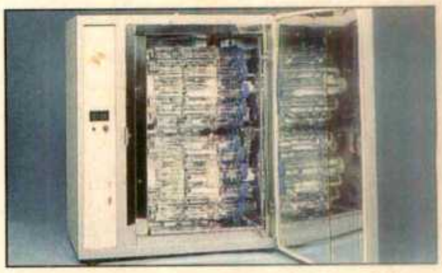

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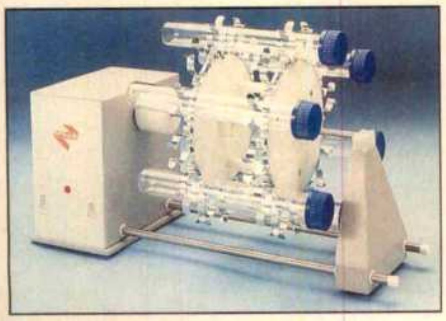

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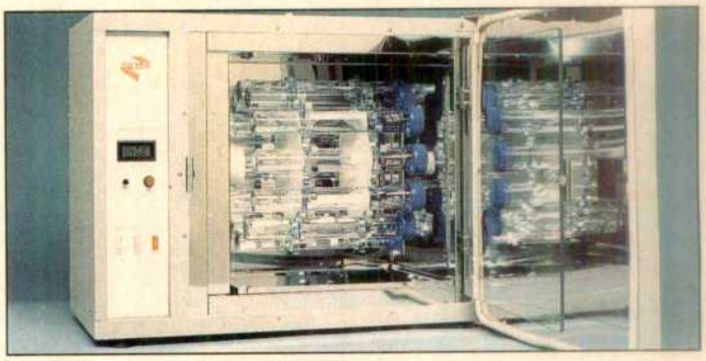

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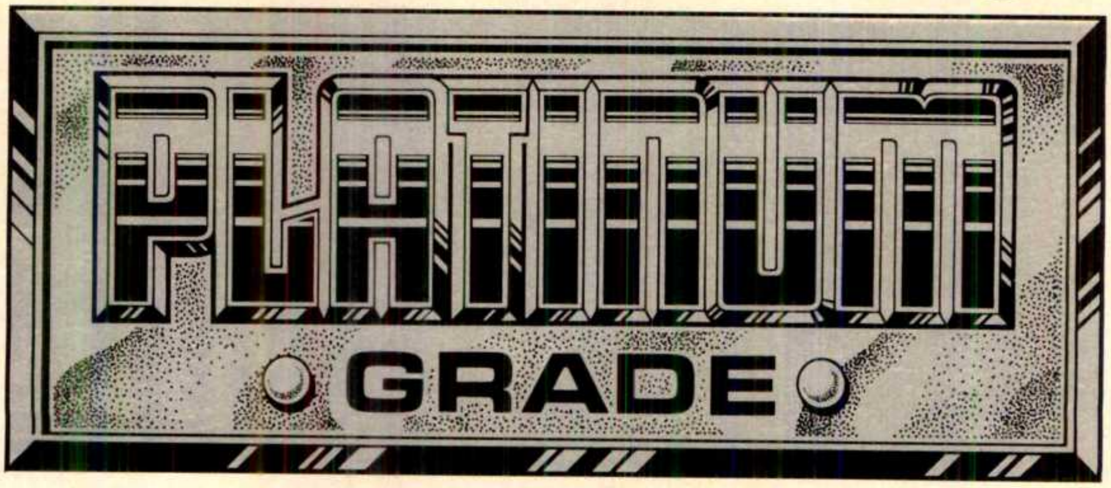

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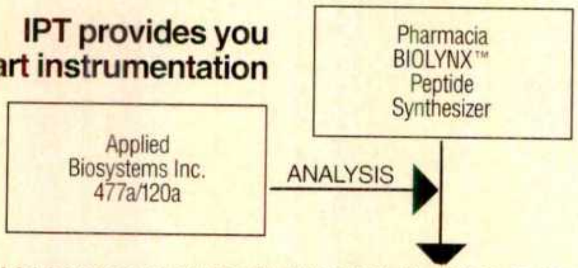

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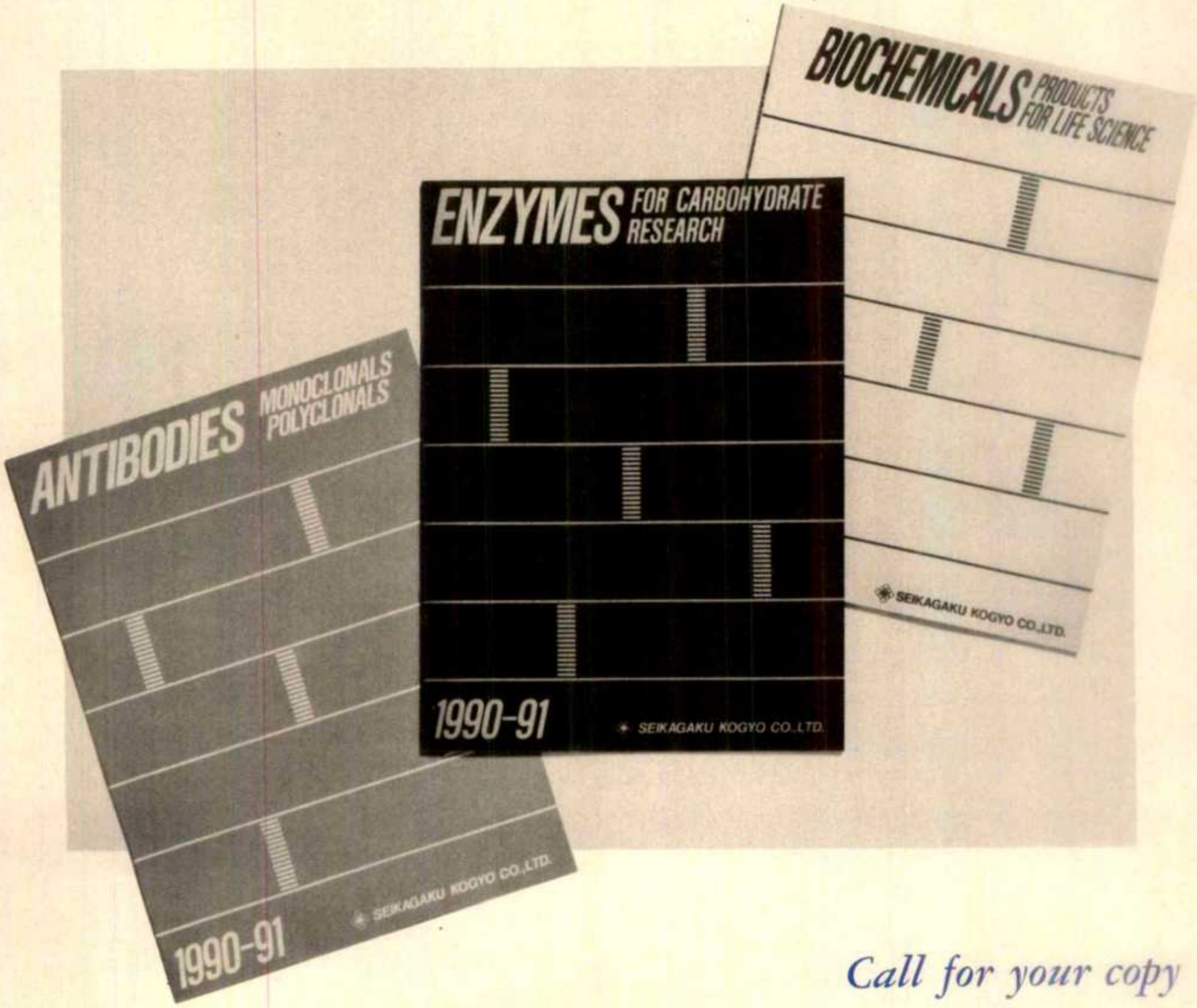

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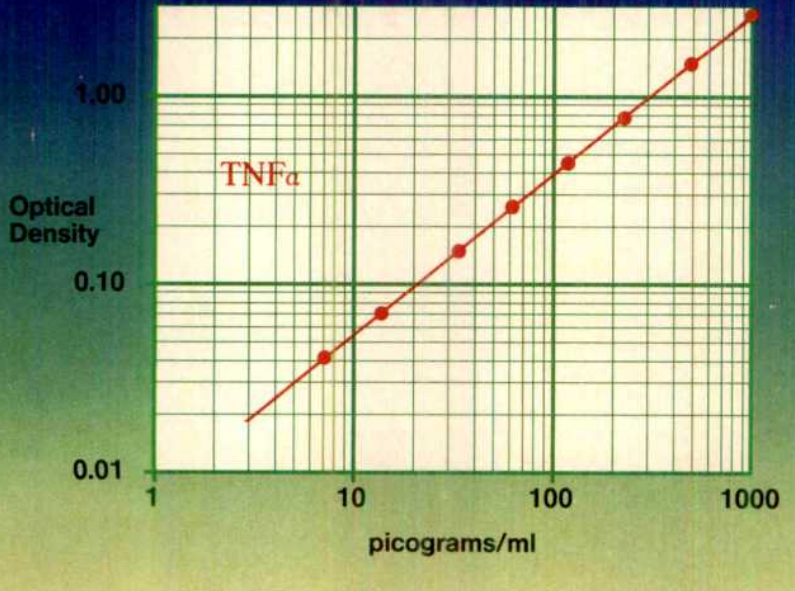

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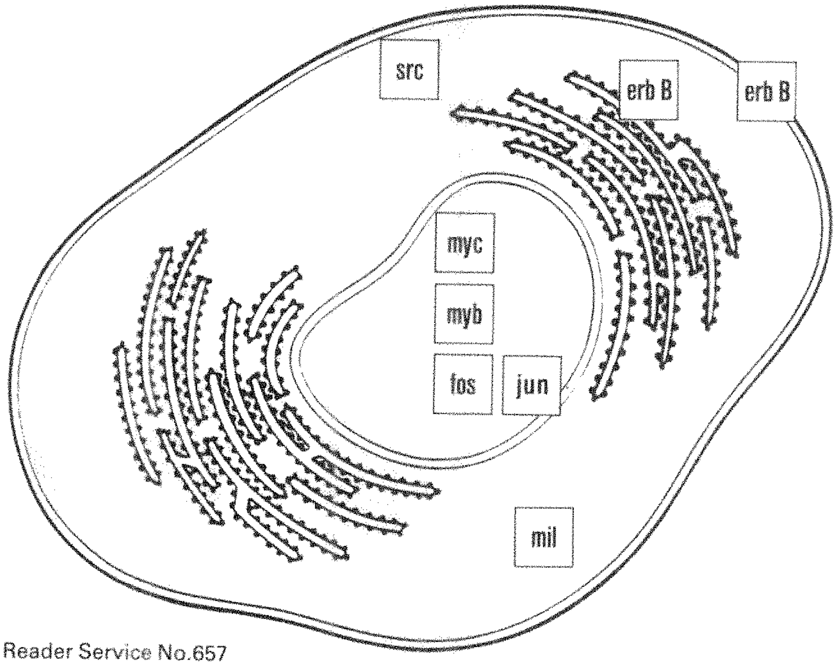

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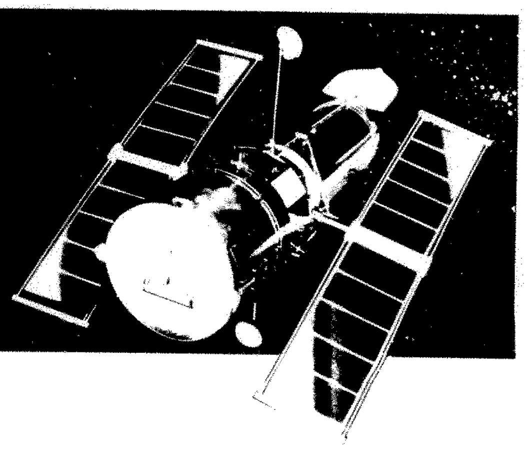

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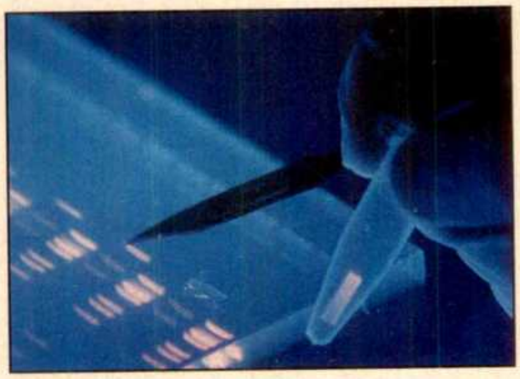

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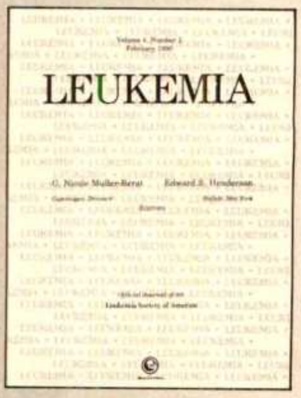

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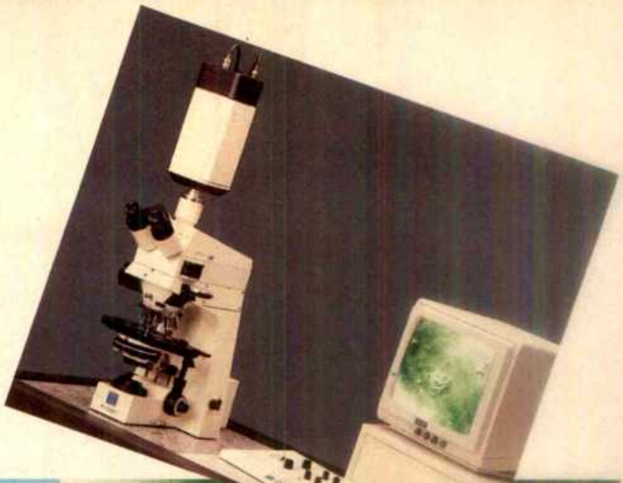

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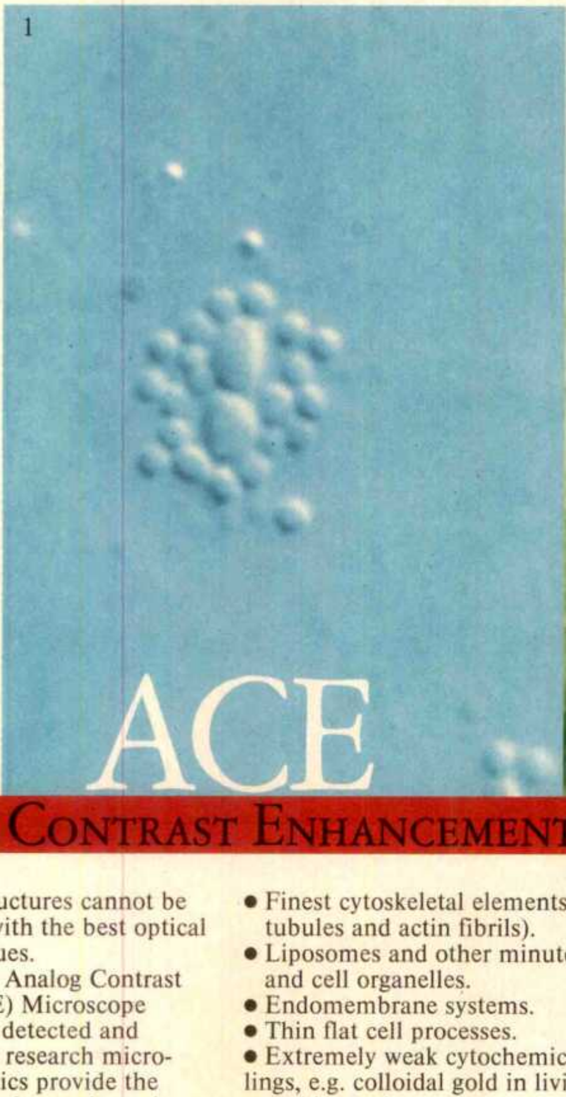

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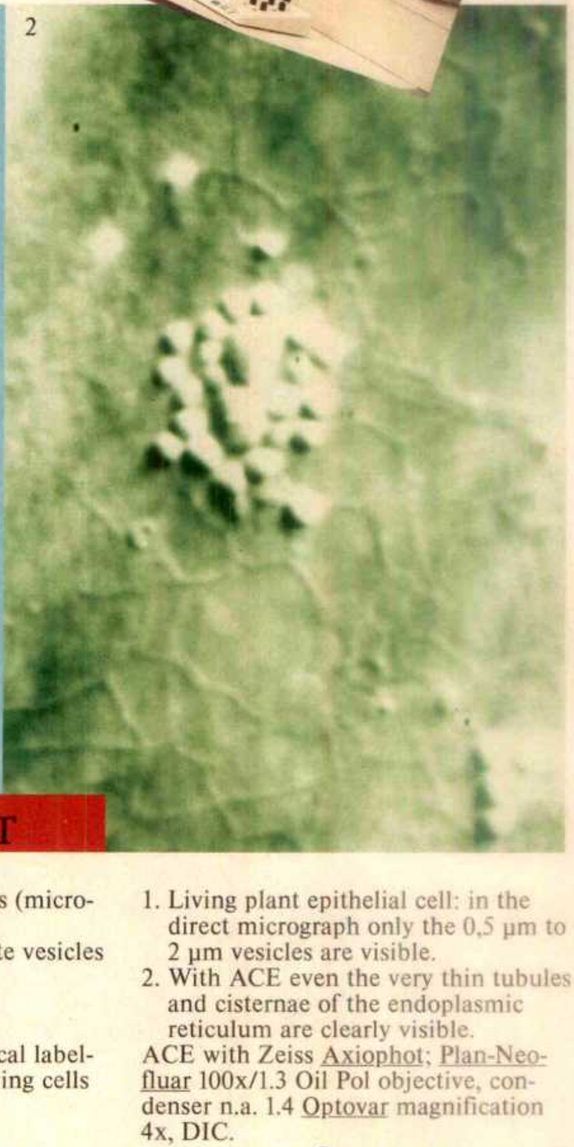

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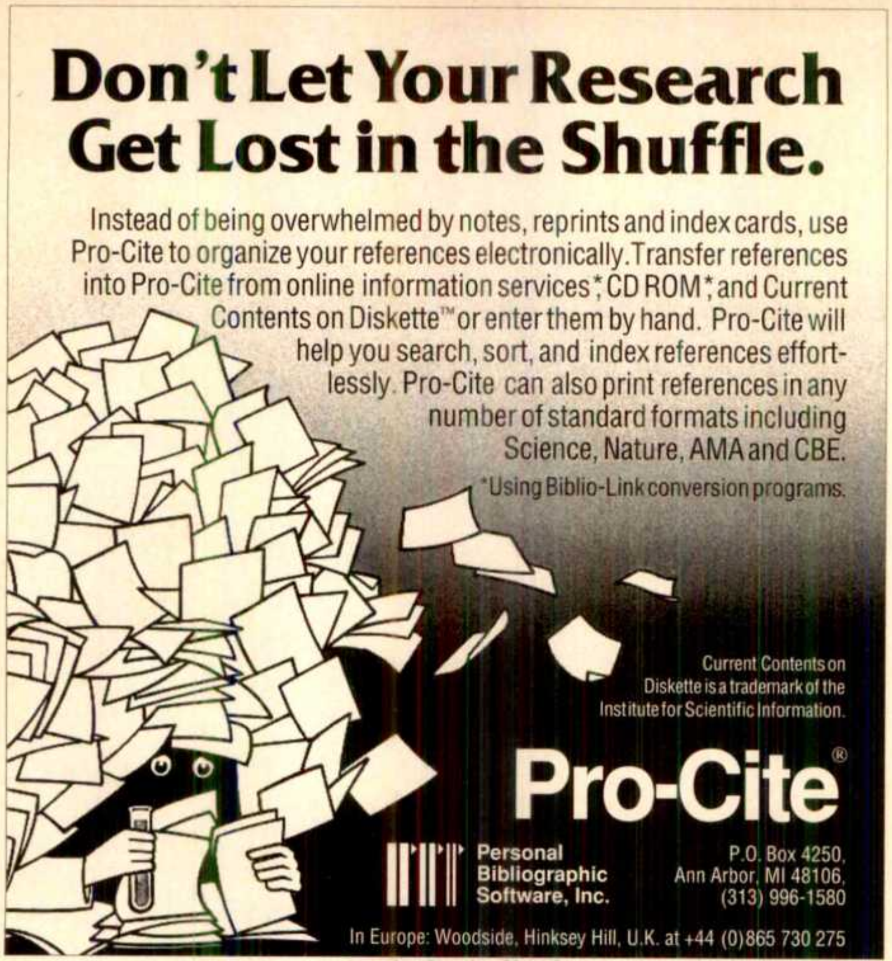

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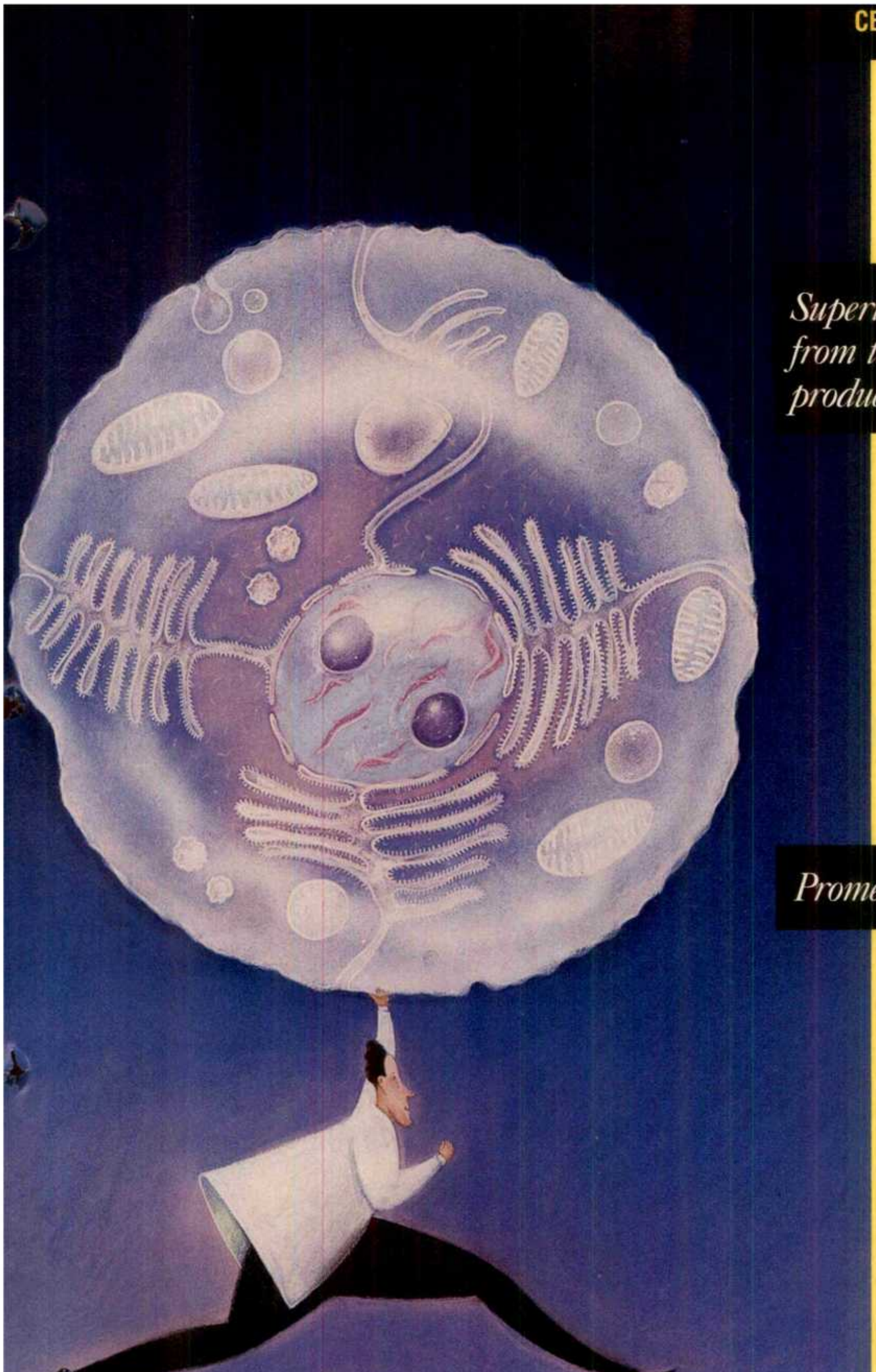

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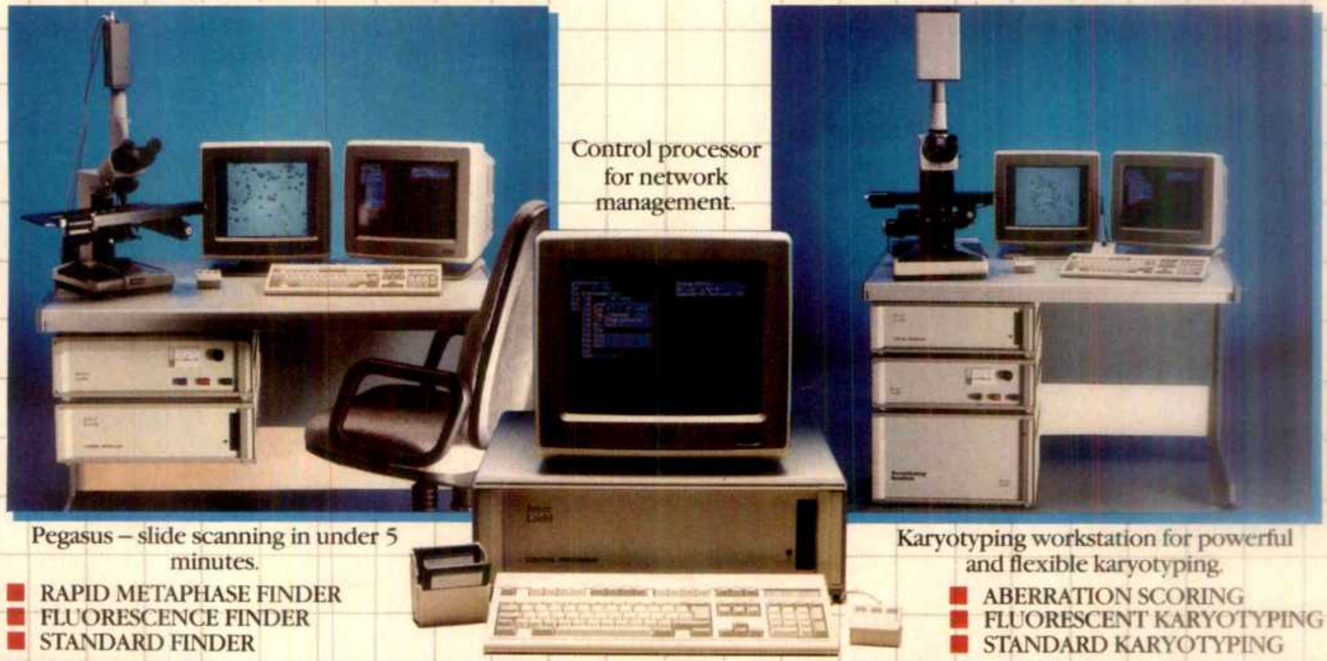

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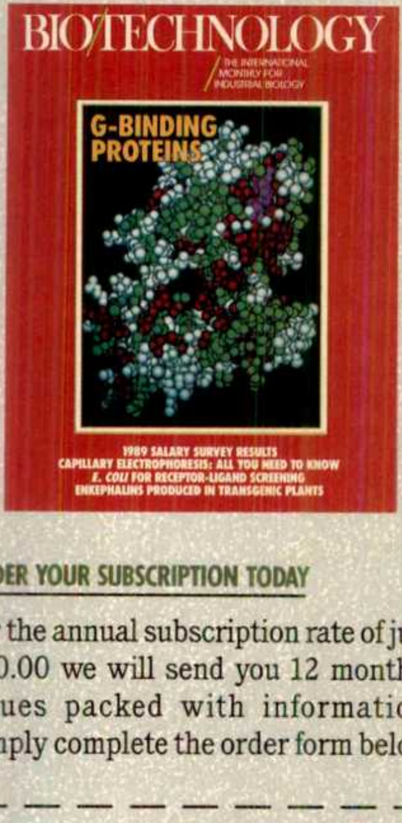
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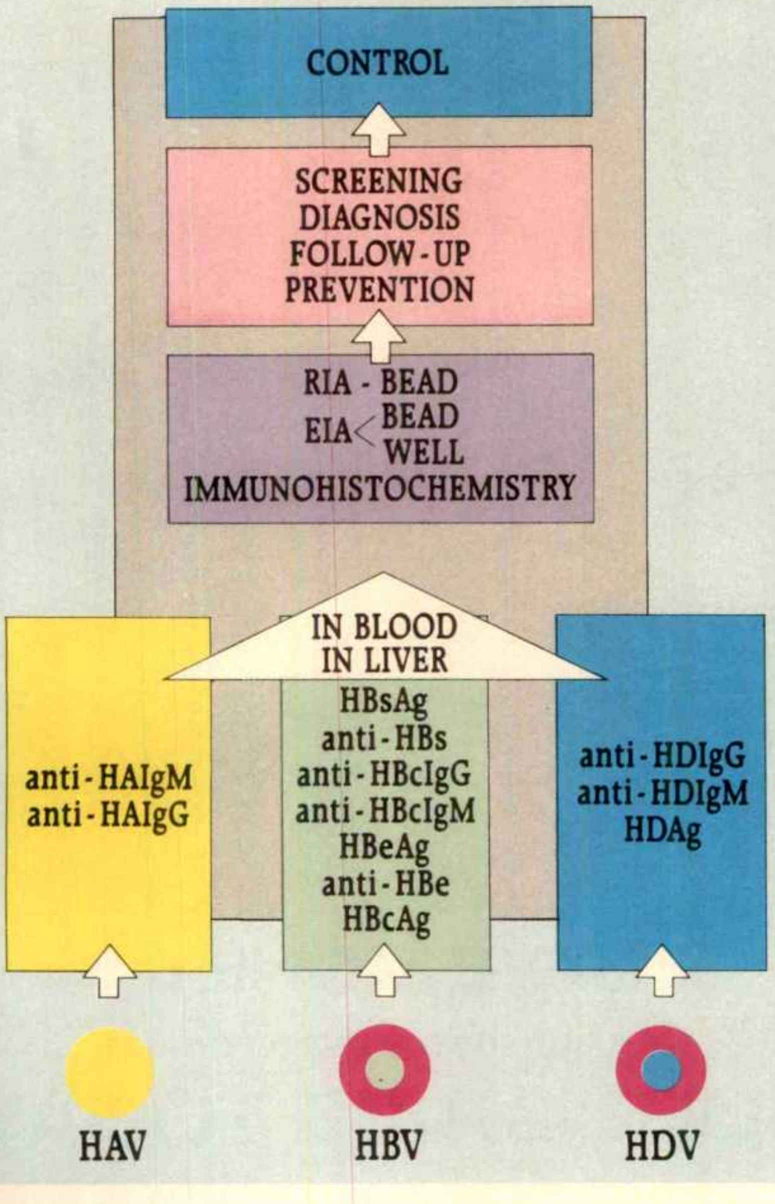

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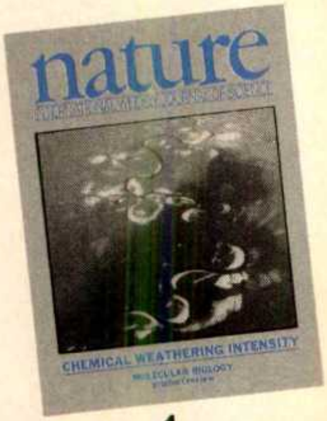

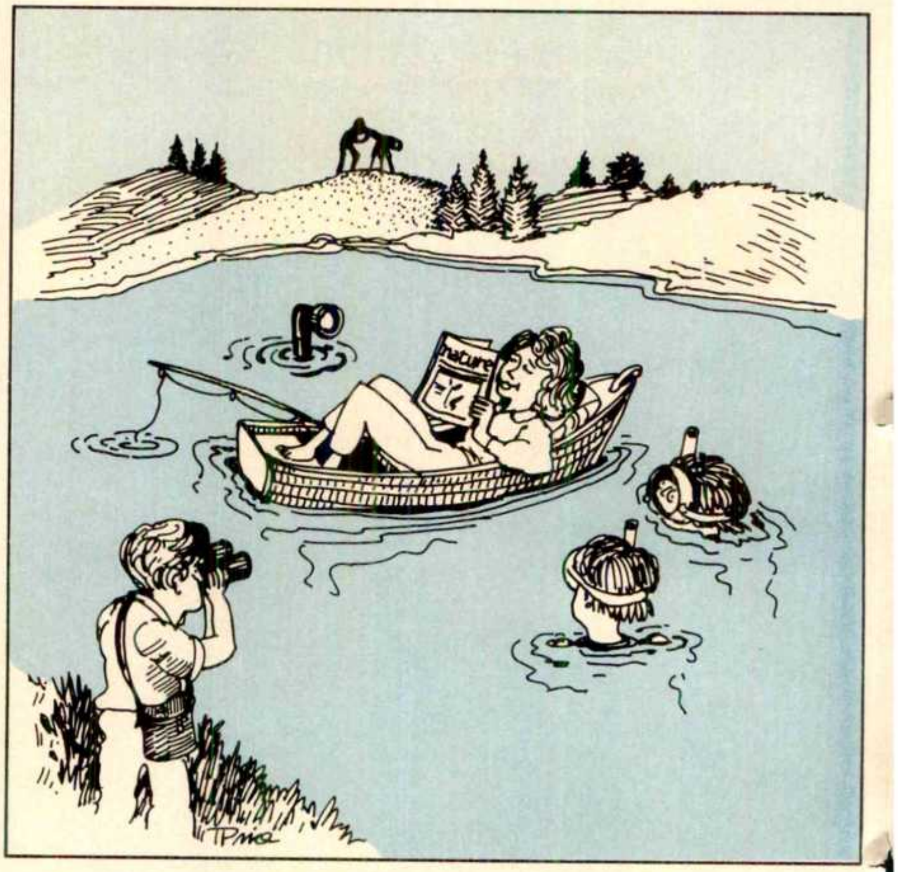

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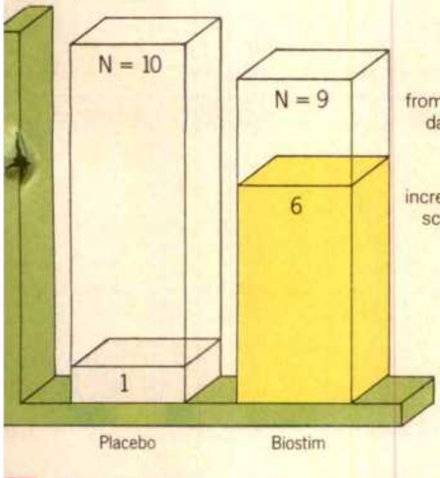

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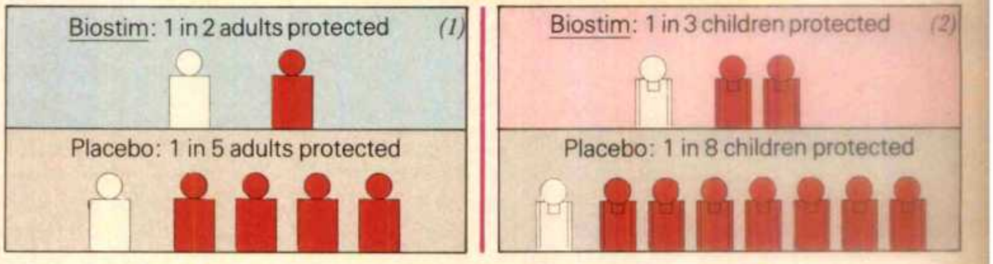

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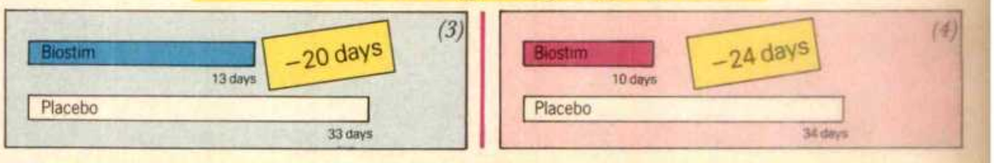

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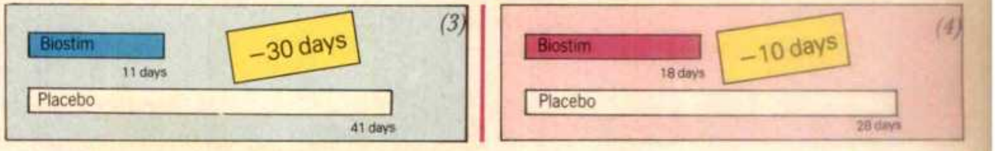

(1) Prof. Chrétien 52 cases — Ref. Rev. Pneumol. Clin., 1985, 41, 3: 213–217
(2) Prof. Pech 78 cases — Ref. Cahiers ORL, 1987, XXII, 3: 217–220
(3) Prof. Boutin 73 cases — Study over 9 months, Ref. Poumon Coeur, 1983, 39: 53–57.
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Mad cows and the minister

A realistic assessment of the risk to human health from bovine spongiform encephalopathy, rather than the hollow pronouncement that "beef is safe" would best counter public fears in Britain.

PUBLIC alarm, even hysteria, has been reawakened in Britain over the possible consequences for human health of the outbreak of bovine spongiform encephalopathy (BSE) in the cattle herd. Several local education authorities have decided to ban British beef from school kitchens. And there are signs that general demand for beef has fallen — to the chagrin of breeders and butchers, wholesale beef prices are tumbling. Agriculture minister John Gummer is under great pressure to do more to protect public health; his difficulty is that, while huge uncertainties persist about the transfer of the BSE agent between species, people included, he is compelled by the nature of his office to provide public reassurance that must seem hollow. His hand may now be forced by the House of Commons agriculture select committee (which savaged the government last year over its handling of a salmonella outbreak in British eggs in 1988).

The origins of the scare over BSE are not far to seek. BSE is the close analogue in cattle of the disease of sheep called scrapie, caused by an infectious agent which is neither a virus nor a bacterium, and possibly a mere polypeptide called a prion. (Those offended by the notion that protein molecules may influence whole cells should concede that regulatory proteins might do just that.) The report a year ago, by a committee under Sir Richard Southwood, of the Department of Zoology at the University of Oxford, suggested that BSE may have arisen in British cattle through the practice, in the early 1980s, of feeding processed sheep offal to cattle, but the evidence for even that inference is circumstantial. Although scrapie is spread in sheep by maternal transmission, there is no evidence of that in cattle. The Southwood committee remarked on the lack of evidence, but was careful not to exclude the possibility. What does seem reasonably certain is that maternal transfer is the only mechanism of transmission of scrapie in sheep, and that in both sheep and cattle, the infection mostly resides in nervous tissue.

People in Britain are not worried about the welfare of cattle, but about themselves and their children. If the scrapie agent has transferred from sheep to cattle, will it now infect people? The discovery that at least one cat in Britain has died of an analogous disease (see *Nature* **345**, 194; 17 May 1990) has triggered the latest alarm by putting in people's minds the notion that inter-specific transfer may be general. If more cats, or animals of other species, die of similar causes, the sense of alarm will certainly be strengthened.

Two difficulties arise. If transmission from cattle to people is possible, and has happened, the disease that will result would probably be indistinguishable from Creutzfeld–Jakob disease (CJD), with symptoms of dementia and characteristic post mortem lesions in the brain. That is why the British agriculture ministry has sensibly commissioned research on the epidemiology of that disease. The snag is that the cause is unknown, but that the incubation-period is long, perhaps 20 years. Those wishing to make precise the risks of present British dietary practice may have to wait that long before they know.

The effectiveness of present precautions in Britain against the transfer of the BSE infective agent from cattle to people is another source of difficulty — made no easier by the latest development reported on page 280 in this issue. The rules are that animals suffering from the disease must be slaughtered and their carcasses destroyed; the brains and spinal cords of other cattle sent for slaughter must be removed by slaughterhouse dissection, and not committed to the human food chain. A certain cynicism inevitably prevails, perhaps fuelled by the observation, earlier this year, that the rate of diagnosis doubled from one week to the next when the British agriculture ministry increased the rate of compensation for slaughtered animals from 50 to 100 per cent. That does not prove that all farmers are venal, but merely that some are capable of self-delusion.

The danger, in Britain, is that the scare will get out of hand. The public worry about BSE is similar to that about the commercial exploitation of nuclear power. The underlying consideration is the welfare (which, loosely, means good luck in the avoidance of death) of individuals in the population at risk. In each case, putatively damaging agents (prions or radioactive substances) are transferred sequentially from one repository to another (from sheep to cattle to people — possibly — or from reactors to the atmosphere to, say, the lungs). The difference is that, by now, the problems of nuclear power are reasonably well understood. The threat from BSE is, by comparison, almost an unknown quantity. What, in these circumstances, should an agriculture minister do and say?

Gummer, the hapless incumbent of the ministry, is not short of advice on what to do. The most radical suggestion, from Professor Richard Lacey of the University of Leeds, is that all animals from herds in which the disease has been found should be slaughtered. That is based on the assumptions either that cattle can catch the disease from those with which they are in contact or that those in the same herds have been fed the same foodstuff. That would entail the destruction of some six million animals. That draconian recipe is politically unacceptable. It is probably also unnecessary. More sensible options for action stem from the supposition that maternal transmission happens in cattle as it does in sheep. The British Veterinary Association was advocating last week that there should be a ban on breeding from the progeny of infected animals, while the British Labour Party went one step further and called for the slaughter of all offspring of BSE cattle. In each case, action is impeded by the length of the incubation period of the disease, and by the lack (in Britain) of a system for tracking the pedigree of cattle. If Gummer wishes to be seen to be acting decisively, he could do worse than take himself to Ireland, which has had a workable system of cattle identity for many years. That, of course, might be demeaning for a British mainland politician.

The economic cost of solutions involving the slaughter of 'at risk' animals is a powerful disincentive. At the current rate of compensation (up to £656 if BSE is confirmed, up to £820 if it is not), Lacey's plan would cost the government several thousand million pounds. Even the Labour party's more modest proposal could run up a bill matching the £6 million paid out to farmers so far. But there is a danger that whatever the British government spends, the economic costs to British farmers stemming from the reduced demand for beef will be greater.

What Gummer should say, as distinct from do, is another matter. Even the agriculture minister's fiercest critics seem glad that it is he, not they who is responsible. But there are a few elementary rules that a man in his position may find useful. First, "don'ts". Never say that there is no danger (risk). Instead, say that there is always a danger (risk), and that the problem is to calculate what it is. And never say that the risk is negligible unless you are sure that your listeners share your own philosophy of life.

The "dos" are philosophically more difficult, but for that reason more important. Gummer should be obliged to tell it like it is. Most probably, BSE would not have arisen if farmers had not been helped to feed processed sheep offal to cattle in the early 1980s. But they were, and the British then paid less for beef than might otherwise have been necessary. Now, with the boot on the other foot, the British will not eat beef for fear that it will kill them, and the price has fallen even further.

Gummer should be saying that predicting such an outturn would have required great feats of imagination. He should also be emphasizing the literally unknown steps in the causation of BSE. An origin in scrapie? The link with contaminated sheep feed is circumstantial only but there are ways in which BSE arising *sui generis* could be worse. Maternal transmission? If scrapie is a guide, maternal transmission is more likely but is similarly unproven and difficult to prove. Adventitious infection? Almost inconceivable, given what little is known of prions, but not entirely out of court. The transfer of BSE to people as CJD? There is not even circumstantial evidence, only hypothesis.

It would not be entirely imprudent for Gummer also to juggle with a few numbers. So far, roughly one in a thousand British cattle have been affected. If the origin is scrapie, and if control measures have been properly applied, a similar number of cattle may be affected in the next five years, after which the disease should disappear. If the transfer of the BSE agent to people from cattle has been contained by the stricter regulation of offal, it is hard to think that people will have been more at risk of acquiring the agent from contaminated beef than cattle have been from contaminated feed. And that risk should now have been eliminated. So the risk that a person eating beef in Britain in the past five years would contract CJD would be between zero (if BSE cannot cause CJD) and, at the most, one in a million. That would make 50 cases of CJD spread over the next 30 years or so, compared with the present case load of about 30 cases a year. Such numbers, only illustrative, might help to take some of the anxiety out of what will otherwise be a bruising debate for all concerned. □

Summit's agenda

Next week's meeting of US and Soviet presidents will be good, but could have been better.

The Washington summit next week will go as well as can be expected. The two superpowers (if that remains the right word) will have made substantial progress towards a treaty on the use of strategic weapons. After fine-tuning on matters such as cruise missiles it will be a valuable piece of paper, when signed later in the year. It is not so much that it will help to reduce the risks of nuclear war, but that it has been made possible by the natural reduction of these risks by other causes. That is worth remembering when both sides set out to tell what difference its treaty may make to their domestic money problems.

The disappointment of next week's summit will be the two sides' failure to reach an understanding on conventional forces in Europe. This has been on the cards since Mr Mikhail Gorbachev's declaration two years ago that Soviet armed forces would be reduced by 500,000. It has been brushed aside by the rapid pace of political change in Europe — notably by the *de facto* collapse of the Warsaw Pact. The Soviet Union is now dragging its feet for one plain reason — it does not wish to get rid of conventional forces while the military status of Central Europe is undeclared — and one dark reason, mounting domestic opposition to Gorbachev's policies. The summit next week could most usefully lay the basis of the long-term security of Central European states shaken loose from the Warsaw Pact. That Europe's most urgent problem is also its most difficult is no excuse for neglecting it. □

MITI maps out the sixth generation computer

■ Fuzzy networks a key component
■ Project may be international

Tokyo

JAPAN's Ministry of International Trade and Industry (MITI) has taken its first tentative step towards mapping out plans for a follow-up to its fifth-generation computer project which ends next year. Details of the new project remain obscure (and in fact are undecided) but it is clear that MITI has set its sights on developing a new generation of massively parallel computers with 'flexible' brain-like information processing systems, including both neuro- and optical computers.

The goals of the new project, which is expected to start in 1992, are described in a report released last month by MITI's Research Committee on New Information Processing Technology, a committee of more than 100 scientists drawn from industry, universities, and MITI's Electrotechnical Laboratory (ETL) in Tsukuba.

Unlike the fifth-generation computer project, which concentrated on the development of a highly parallel computer based on existing predicate logic, a key aim of the new project will be to lay down new theoretical foundations for parallel computing. Shunichi Amari of Tokyo University, who developed some of the mathematical theory behind neural network models in the 1960s and 1970s, is an adviser for the project and a strong advocate of such theoretical work.

The theoretical part of the project will set out to establish 'soft (flexible) information processing systems' capable of handling ambiguous or incomplete information in the form of patterns or symbols as well as numbers, with the capacity of learning and self-organization, and that can deal with "approximately correct problem solving" and the "integration of mass information". The research will include and expand on the theory of fuzzy computing and neural network processing, according to Taizo Nishikawa, deputy director of MITI's industrial electronics division.

On the technological side, the project will develop massively parallel computers by looking at various types of new devices, computer architecture and software. The committee has not set its sights on a specific type of parallel computer.

The report includes diagrams of an optical digital computer and a picture of a small piece of hardware that contains one thousand million 'neurons' and a hundred million million transistors on 8-inch wafers stacked 50 centimetres high on a board only one metre square (the committee sees wafer-scale integration as one possible route to the development of massively parallel computers). But with a power consumption of 160 kW, cooling such a pile of hardware will be just one of many technical problems to overcome.

Although MITI officials are not prepared to comment on the size of budget for the project, it is widely expected that it will be similar in scale to the fifth-generation computer project which will have consumed about ¥50,000 million ($325 million) over ten years by the time it ends next year.

ETL researchers who were involved in planning both projects are determined that the new project should not be organized along the same lines as the fifth-generation computer project. Their reason is simple. ETL did not get a penny out of the fifth-generation computer. (Similarly, people in industry are not happy because their best people have been tied up in the project for small financial returns.) ETL belongs to MITI's Agency of Industrial Science and Technology. But the fifth generation computer project comes under the jurisdiction of MITI's Machinery and Information Industries Bureau (to which Nishikawa's industrial electronics division is attached). Such is the internal bureaucracy of MITI that despite early attempts ETL could not receive any fifth-generation computer funds.

One way to surmount infighting might be to make the new project international. Nishikawa in fact says that it is "imperative" that the project be international, and he and some of the committee members have already visited government officials and academics in the United States and Europe to explain the goals of the new project. Nishikawa hopes to include foreign representatives (from both government and industry) who will be based in Tokyo as many of the present committee members would prefer to communicate in Japanese.

Nishikawa expects that the new international planning committee will hold its first meeting in June or July. The main aim of the committee will be to decide how the goals mapped out in last month's report can be achieved. Next year the project will enter the 'feasibility study' stage and although Nishikawa is not prepared to comment on the budget for fiscal year 1991, ETL researchers expect the project to get several hundred million yen (a few million dollars). **David Swinbanks**

ENVIRONMENT

Compromise at Bergen conference

London

ALTHOUGH dismissed as vague by environmentalist groups, the ministerial declaration from last week's conference on sustainable development in Bergen, Norway, should help progress towards the more definite international agreements on environmental protection expected to be reached at the global United Nations conference in 1992.

In line with Norwegian proposals, delegates agreed that lack of "full scientific certainty" over threats to the environment should not postpone preventive measures. But, at the insistence of the United States (see *Nature* 345, 193; 17 May 1990), commitments to rigid targets for reduction of carbon dioxide emissions and for financial aid to developing countries to phase out chlorofluorocarbons (CFCs) were excluded.

Ministers from 34 countries (including Western and Eastern Europe, plus the Soviet Union, the United States and Canada) agreed to draw up national strategies for carbon dioxide controls after they receive the report that will soon be completed by the Intergovernmental Panel on Climate Change (IPCC). The report will be considered next week by the scientific working group of IPCC at a meeting near Windsor in the United Kingdom. A draft of the report has already been leaked to the media (see *Nature* 344, 577; 2 April 1990). The Windsor meeting will be followed in the next few weeks by meetings of the working groups on the impact of climate change and policy to combat climate change in Moscow and Geneva.

UK environment minister David Trippier, attacked before the conference over British support for the US position on carbon dioxide emissions, said the United Kingdom will establish its national strategy before the world climate conference in Geneva in November. But he warned that solving the greenhouse problem could cause "pain and anguish" to the British electorate.

At Bergen, the United States was once again attacked for its reluctance to help developing countries meet the costs of using alternatives to CFCs, just as it was at the Geneva conference on CFCs earlier this month (see *Nature* 345, 193; 17 May 1990). The Bergen declaration calls on the Montreal Protocol meeting in London next month to strengthen international action to protect the ozone layer, but inserts "for example" before the key phrase "through additional resources and technology transfer".

Observers hope the compromise wording will avert the threatened Indian boycott of the London meeting. India and many other developing nations have not yet signed the Montreal Protocol. Peter Aldhous

BSE

New fears on transmission

London

THE UK agriculture ministry's Central Veterinary Laboratory in Weybridge, Surrey, is examining a suspected case of bovine spongiform encephalopathy (BSE) in a cow born after the government banned the use of cattle feed containing sheep and cattle offal. If confirmed, the case will embarrass the UK government, which maintains that the ban prevented any further spread of the disease.

The feed ban came into force in July 1988 after scrapie-infected sheep tissue was identified as the probable source of the BSE outbreak. Confirmation of the new case (believed to be a 15-month-old cow from southern England) may mean that the BSE agent can be transmitted from infected cows to their offspring, probably via the placenta, just as scrapie can be 'vertically transmitted' in sheep. But violation of the feed ban by a farmer left with stocks of feed containing sheep offal is another possible cause. If vertical transmission is to blame, then more cases of BSE in young cows should emerge over the coming months. Fears of vertical transmission of BSE lie behind calls for a ban on breeding from the offspring of animals that suffered from BSE.

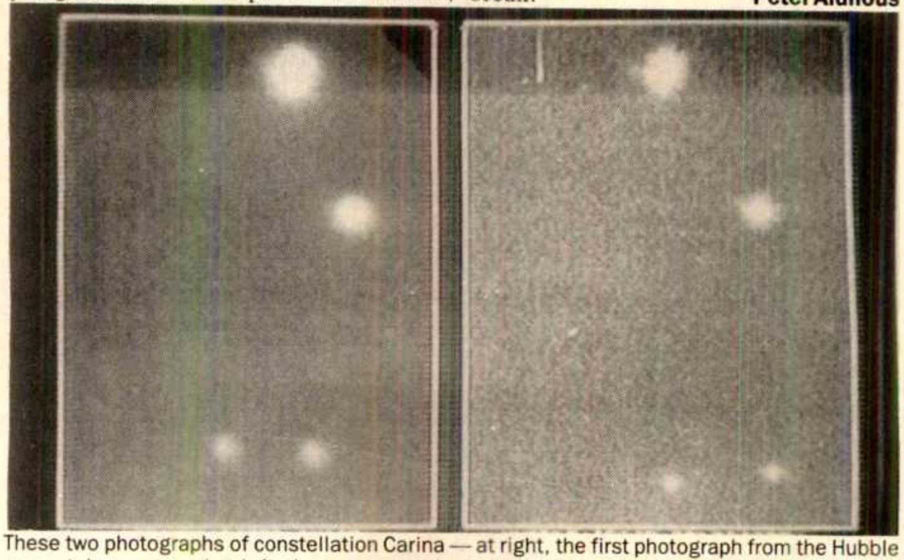
Francis Anthony, chairman of the British Veterinary Association's farm animals committee, says that he supports a breeding ban, not on the grounds of public health, but to help the British beef industry. If herds are bred that have no connection with the BSE outbreak, he says, then British beef may regain some lost export markets. But a breeding ban may be limited in its effect: accurate pedigrees are not kept for all British cattle, and many cows may be infected that do not yet show symptoms.

Anthony estimates that vertical transmission of BSE could delay the eradication of the disease from British cattle by 20 years. Even without vertical transmission, most experts expect new BSE cases to arise until the end of the century, because of the disease's long incubation period.

Anthony believes that the imposition of a breeding ban could give Britain a head start in dealing with BSE, as he thinks it likely that outbreaks will soon be confirmed in other countries (see *Nature* **344**, 805; 26 April 1990). In the United States and France, BSE cases may be dismissed as rabies, Anthony says. The United States Department of Agriculture (USDA) seems to share his concern. Gary Colgrove of USDA says that a project has just been begun to examine the brains of cattle with unidentified neurological disease. Those suspected of containing lesions caused by BSE will probably be sent to the United Kingdom for further examination.

The current uproar surrounding BSE in the United Kingdom was sparked off by the discovery of spongiform encephalopathy in a domestic cat from Bristol (see *Nature* **345**, 194; 17 May 1990), which reawakened fears of transmission to humans. A second suspected case, in a cat from Northern Ireland, is now being examined. Agriculture minister John Gummer, facing growing criticism from the British press, appears before the House of Commons agriculture select committee this week to explain the British Government's action to control the BSE outbreak.

Peter Aldhous



These two photographs of constellation Carina — at right, the first photograph from the Hubble space telescope, and at left, the same star cluster taken by the Las Compaños Observatory in Chile — were released by the US National Aeronautics and Space Administration (NASA) last week. What appears to be an elongated blur at the top of the left photo has been resolved into a double star by Hubble. Although the first Hubble pictures were several times sharper than expected, NASA engineers expect to be able to improve Hubble's resolution considerably when the telescope is fully tuned up later in the year.

BIOTECHNOLOGY

Health worries over use of milk hormone

Washington

COMPLAINTS by small dairy farms that a genetically engineered hormone designed to boost milk production could put them out of business has led two US states temporarily to ban the product.

Last month the governors of Minnesota and Wisconsin signed legislation that places a ban until mid-1991 on the sale or use of bovine somatotropin (BST) in the two states, which, combined, produce 20 per cent of the United States' milk. Wisconsin Governor Tommy Thompson said that the ban would allow "additional farmer and consumer education" without hurting the state economically. Vermont's legislature last week rejected a similar proposal.

Opponents of the hormone claim that it encourages overproduction, which would flood the market and eventually drive out small dairy farmers.

Health worries remain an issue in the debate. Although the US Food and Drug Administration (FDA) says that its studies show that the hormone presents no danger to the public, it has not yet published the data behind that claim.

The US General Accounting Office is investigating charges of favouritism in the FDA's examination of the drug. Jeremy Rifkin, president of the Foundation for Economic Trends, a Washington-based group that opposes BST and some other biotechnology products, points to several studies that raise questions about the hormone's biological impact. Beyond any possible impact on humans, BST appears to shorten the life of the cow. Although milk production can rise 10 to 25 per cent, "cow burnout" can cut reproductive lifespans by a third, he says.

Four biotechnology companies — Monsanto, Eli Lilly, American Cyanamid and Upjohn — have spent more than $500 million in developing the drug. But BST has been plagued by public opposition and controversy since field trials began in 1985. Last year 2,500 US supermarkets agreed to boycott BST-produced milk (*Nature* 340, 667; 31 August 1989). Until FDA approves BST for general sale and use, which the agency plans to do later early next year, the supermarkets say they will not sell the milk.

Although the BST bans appear to place yet another hurdle in the path of agricultural biotechnology products, analysts caution against extrapolating. "It's one specific product. We don't think it means big trouble when you look at all the other [agricultural] biotech products", says Alan Goldhammer of the Industrial Biotechnology Association.

G. Christopher Anderson

SUPERCONDUCTIVITY INDUSTRY

Argonne treads carefully

Washington

In an arrangement carefully crafted to avoid charges of federal meddling in industry, an investment fund set up by the Argonne National Laboratory and the University of Chicago has been used to start a company to market the laboratory's superconductivity technology. Officials say the arrangement marks the first time a US government laboratory has become involved in the creation and management of a private company.

Known as the Illinois Superconductor Corporation (ISC), the start-up company obtains one third of its initial $1.5 million funding from the ARCH Development Fund, a venture-capital fund started by the laboratory and the University of Chicago, which runs Argonne for the Department of Energy. Additional funds come from the state and a private venture capital firm. The company is the first Argonne spinoff created by ARCH.

Although laboratory officials are careful to emphasize that federal dollars are not going directly to the company, but are filtered through ARCH, the agreement is reminiscent of a deal that lost the chief of another federal agency his job last month. Craig Fields, head of the Defence Advanced Research Projects Agency (DARPA), was removed from his post after deciding that the agency should invest $4 million in Gazelle Microcircuits Inc., a small semiconductor company.

For the conservative Bush administration, which favours free markets and resists government intervention in industry, Field's move smacked of an "industrial policy" by which the government would decide to support one technology or company over another.

In the case of Gazelle, the technology was fast gallium arsenide microelectronics, a difficult but promising technique that has had trouble attracting traditional investors. By becoming a partner in Gazelle, DARPA — and the federal government — effectively became a very large and potentially unfair competitor in the industry.

What makes the Argonne start-up different, says ARCH director Steven Lazarus, is that the laboratory is driven solely by a wish to get its technology into the field, rather than the urge to support an ailing part of the industry. Six Argonne superconductor inventions will go into ISC's first product, a sensor to measure the temperature of refrigerants. Because the independently run ARCH — and not the laboratory directly — is the actual partner in the new company, other companies are unlikely to complain that they are forced to compete against the US government, Lazarus says.

Argonne puts $200,000 a year into ARCH to find outlets for the laboratory's technology. If ISC becomes successful, a share of its profits will return to ARCH, which will then return funds to Argonne's technology transfer programme to encourage further spinoffs.

G. Christopher Anderson

BIOTECHNOLOGY INDUSTRY

Japanese/US joint venture announced

Washington

Consolidation within the biotechnology industry continues apace with the announcement this week that US-based Genetics Institute is entering into a joint venture with Yamanouchi Pharmaceutical Company Ltd, one of Japan's largest drug companies. The two-part agreement involves the formation of a separate company for the commercialization and marketing of Genetics Institute's genetically engineered bone morphogenetic proteins (BMPs) in Japan, and the setting up of a partnership to support the development and commercialization of BMPs worldwide.

As a result, Genetics Institute is likely to become the first US biotechnology company to establish a direct marketing presence in Japan. In the carefully crafted agreements, Genetics Institute will retain worldwide manufacturing rights and US marketing rights to its BMP products, and will receive a 50 per cent share in the profits from the sale of its BMPs in Japan.

Gabriel Schmergel, president and chief executive officer of Genetics Institute, expects that by the late 1990s, up to one-third of Genetics Institute's business could be in the bone area, worth in excess of $2,000 million worldwide. Bone morphogenetic proteins have been shown to induce new cartilage and bone formation. They have potential as a substitute for existing bone graft materials in fracture healing and in the treatment of bone loss associated with periodontal disease and certain cancers.

BMPs are in the pre-clinical testing phase of development and products are not expected on the market for some years.

Genetics Institute has forged close business ties with Japanese companies — Chugai Pharmaceuticals and Suntory as well as its new joint venture with Yamanouchi. "All of our past deals in Japan were licensing deals — everything else will now be channelled through this joint venture, of which we own 50 per cent", says Schmergel.

Diane Gershon

INFORMATION FLOW

Soviet doors opening up

Leningrad

It has been cogently argued that of the many frustrations to research scientists in the Soviet Union, the worst is the sluggish flow of information from elsewhere: in a fast-moving field, not to know what others have already done, or tried and failed to do, is crippling. While the post, telephone and fax on the whole serve Soviet scientists rather ill, perhaps the most important device for the transmission of information is the international conference. So it is a significant achievement of the Institute of Cytology of the Academy of Sciences of the USSR to have brought to Leningrad last week some two dozen biologists from the United States, Europe and Japan (but mostly from the United States) to speak, along with about a dozen Soviet participants, on the highly topical theme of chromosome transmission and mitosis.

The idea for the conference seems to have arisen during a year-long visit by one of the organizers, Natalay Kouprina (Institute of Cytology, Leningrad) to the laboratory of co-organizer Phil Hieter at Johns Hopkins University in the United States; other members of the organizing committee were Vladimir Larionov and N. Nikolski at the Institute of Cytology, and William Earnshaw at Johns Hopkins.

Scientifically, the conference marked the early stages of a new assault on the macromolecular miracle whereby each time a eukaryotic cell divides, its microtubules assemble into a mitotic spindle that captures the paired and duplicated chromosomes and delivers one set reliably to each daughter cell.

Two traditional lines of attack on the problem may be expected to converge: many participants reported the identification of mutant yeast genes that affect the mitotic machinery so as to increase chromosome loss; many others contributed animal proteins that bind to the chromosomes themselves, or to the spindle microtubules, or both, and are thereby implicated as components of the machinery. The expectation is that some of the animal proteins will turn out to be the products of the genes defined in yeast.

The conference provided a forum for discussion of a central topic in cell biology at an exciting phase of its development when much of what is happening is not yet in print (but see *Nature* **345**, 22 & **345**, 206; 3 & 17 May 1990). This is the sort of thing Soviet scientists need. But it cannot have been conducive to Soviet participation in informal discussion that, in the retreat to the hard-currency hotel bar each evening, local participants were put in the invidious position of having to accept the hospitality of their guests.

Miranda Robertson

OTA REPORT

Neurotoxicology in need of a fix

Washington

US research into the neurotoxic effects of hazardous chemicals is fragmented and inadequate, concludes a report released last week by Congress's research body, the Office of Technology Assessment (OTA). As a result, illness stemming from exposure to neurotoxic substances may often go undetected.

Among the options the report offers are substantial increases in support for research and screening of potentially harmful compounds, increased support for neurotoxicology training programmes and a five-year plan to boost the impact of research supported by the National Institutes of Health (NIH).

OTA found the programmes currently carried out under the auspices of NIH, the Environmental Protection Agency and the Alcohol, Drug Abuse, and Mental Health Administration to be of "insufficient size and scope" to address the full problem of environmental neurotoxins. The report stresses that EPA's intramural neurotoxicology programme has been hampered by lack of funding. Despite the need for effective *in vitro* neurotoxicity tests that will minimize the use of animals, EPA recently had a request for extra funds to develop such tests turned down by a government budget office.

The new report emerges against a background of increasing concern over the neurological effects of chemicals. In a series of influential studies, William Langston and colleagues at California Parkinson's Foundation found that MPTP, a chemical created as by-product during the illicit manufacture of synthetic heroin, can induce the symptoms of Parkinson's disease. MPTP's development as a herbicide had previously been halted only by the emergence of more effective chemicals. Langston now believes that the "preponderance of the evidence" indicates that Parkinson's disease is largely caused by non-genetic factors, although others still think there may be a significant hereditary contribution.

Also mentioned in the OTA report is evidence suggesting that a recent increase in the incidence of motor neuron disease, primarily amyotrophic lateral sclerosis (ALS), in the United States is due to environmental agents. But establishing the identities and biochemical effects of the causative agents is likely to prove difficult. The report claims that differences in scientific judgement on the general role of environmental factors in diseases such as ALS and Alzheimer's disease, as well as the parts played by particular causative agents in the progress of neurological diseases, hamper the coordination of federal research and regulatory programmes.

David Concar

GORE-TEX

Gore weathers storm

Washington

The latest in a series of sporadic legal wrangles over the true identity of Gore-Tex — a stretched form of the low-friction plastic Teflon which is widely used in outdoor clothing as well as such things as space suits and artificial arteries — has ended with the US company that discovered the material coming unstuck over a vaguely worded patent and evidence of an earlier discovery in Japan.

The company, Maryland-based Gore Associates Ltd, has long weathered legal storms over Gore-Tex, but last week showed signs of losing its grip on the material when a US federal court in Phoenix rejected the company's claims of patent infringement by Impra, a medical prosthetics company in Tennessee. Judge Charles Hardy, ruled that Gore's patent specifications failed to define a unique material and so offered no protection.

Gore-Tex was discovered serendipitously by Robert Gore in 1969 when in stretching a rod of polytetrafluorethylene (PTFE) — the plastic from which Teflon is made — he inadvertently gave it a sharp tug rather than a slow pull. The resulting material was an expanded form of PTFE that combined the chemical inertness and low-friction properties of Teflon with high tensile strength and permeability.

What began as a small basement business is now an international concern with a reputed turnover of more than $700 million. Yet Gore's success belies a host of patent problems. It took three failed applications and a delay of ten years before the company succeeded in obtaining a US patent in 1979. And in 1984, the company came close to losing that patent in a suit against a New York company called Garlock Inc. Throughout its patent wrangles, Gore has been dogged by questions about an earlier Japanese patent issued to Sumitomo Electric Industries Ltd in 1967. In 1984, Garlock Inc. tried but failed to convince an appeals judge that the materials specified by the Japanese and Gore patents were indistinguishable, and that the later Gore patent was therefore invalid.

Gerald North, Impra's attorney and a veteran of the 1984 dispute, believes that one reason why Impra has succeeded where Garlock failed was that Impra commissioned a team of distinguished materials scientists — led by Edward Clark of the University of Tennessee who pioneered the early studies of PTFE in the 1950's — to make the Japanese material, following the patent specifications down to the last detail. The result, says North, was a plastic which, when tested according to Gore's own protocol, proved indistinguishable from Gore-Tex.

Gore's attorney, Iain Campbell, however, maintains that the prior Japanese patent is "hopelessly vague" and that it was only with the "hindsight" afforded by the later Gore patent that Impra's scientists were able to use it as a recipe for Gore-Tex.

A second factor crucial to Impra's victory was the judge's ruling that Gore's patent fails to define which of several possible tests should be used to characterize the material's tensile properties. "Gore had provided in effect no way to determine whether infringement had or had not occurred in a particular case", says North. But Campbell argues that the ruling on tensile strength tests reflects a "wooden interpretation" of the patent and that it would be obvious to an expert which of the tests should be used.

Gore-Tex intends to challenge the ruling in the Courts of Appeal. And according to Campbell, if the ruling is upheld on appeal it could have general implications for the way in which patents for new materials are written in the future.

David Concar

ARIANE

Back up by August

Paris

The European commercial space rocket, Ariane, should resume launches at the end of July or the beginning of August, according to Frédéric d'Allest, the director of Arianespace. Launches were stopped pending an inquiry after the last launch, flight 36, ended in an explosion a few minutes after lift-off. As the cause of the explosion was a discarded rag blocking cooling circuits, safety checks have been stepped up, but the design of Ariane rockets has not needed to be revised.

Customer confidence in the launcher has evidently not been shaken either — six new contracts have been signed since the explosion. The next launch will carry the French direct-broadcasting television satellite, TDF-2, and DFS-2 Kopernikus, a West German telecommunications satellite.

Peter Coles

FAST-BREEDER REACTORS

Superphénix encore

Paris

The French fast-breeder reactor, Superphénix has developed a new leak in its liquid sodium cooling system. At fault is a junction in heat-exchange circuitry, and about 10 litres of coolant are said to have been lost during the night of 27 April.

The reactor has been shut down while tests are carried out for other cracks and is expected to restart in June. The reactor, for whose closure neighbouring Swiss residents have been campaigning for several years, developed a serious leak in 1987 and was out of service for two years.

Peter Coles

INDOCHINA

Cambodia's killing fields

Annapolis, Maryland

TEN years after the Khmer Rouge were driven from Phnom Penh, rice research and production in Cambodia remains a victim of the Pol Pot regime, Glenn Denning of the International Rice Research Institute told a Rockefeller Foundation agricultural research conference last week.

Among the scars left by the Khmer Rouge attacks of the early 1970s are burned rice-research stations like the one shown here. As the Khmer Rouge first advanced towards Phnom Penh, Cambodia's rice farmers abandoned their crops to flee to the cities. By 1975, when Phnom Penh fell, rice-growing area in Cambodia was a fifth of the 2.5 million hectares cultivated five years earlier, Denning said.

In Cham Car Daung, near Phnom Penh, the Khmer Rouge took over the Institute of Agriculture, the country's main agricultural research university. It was converted into an ammunition dump by the Pol Pot regime, then blown up on their departure in 1979. Of the 300 graduates from the institute's heyday between 1969 and 1975, only 20 have been accounted for. The rest have either 'perished' or left the country, Denning said.

To convert Cambodia to irrigation, Pol Pot banned traditional deep-water rice and commissioned massive networks of waterways. 'Pol Pot canals' still mark much of the country, but few are functional. Those that are still maintained are used as fish ponds.

During the Khmer Rouge attacks, hungry Cambodians ate their seed rice, losing the ability to plant again. With the lost fields went hundreds of unique rice species. "It appeared that centuries of selection had been lost," Denning said.

The legacy of the seventies.

Since 1981, shortly after the first relief workers arrived in Phnom Penh, the International Rice Research Institute (IRRI) based in the Phillipines has reintroduced 562 traditional species to Cambodia, Denning said. Half of the rice now grown in Cambodia comes from species taken from IRRI seed banks. IRRI currently has three scientists stationed in Phnom Penh, including a anthropologist to study traditional farming methods.

After a decade of collective farming in 'crop solidarity groups', Cambodian farmers were last year given the option of 10–20 year leases on the land they worked and were allowed to market their own crops. Although prices of both rice and fertilizer have subsequently gone up, so has production. Rice-growing land now accounts for 1.8 million hectares. Even so, average yield is only 1.2 tons per hectare — still the lowest in Asia.

G. Christopher Anderson

PASTEUR INSTITUTE

Revival of links with Romania

Paris

AFTER 15 years of imposed isolation, the Instituto Cantacuzino in Bucharest has been able to renew contact with the Pasteur Institute in Paris, the institute upon which it was originally modelled. A package of initiatives worked out by the Pasteur Institute aims to bring researchers up to date in the latest techniques of molecular biology and modern virology and to help put the Institute back on its feet.

Set up in the 1920s, the Instituto Cantacuzino should be playing a central role in efforts to control the spread of hepatitis B and AIDS. But after a decade without access to international science, either through the literature or through travel, researchers have been left far behind.

Training will be offered in Paris to Cantacuzino's best researchers and a 3-week summer school organized in Romania, for about 20–25 researchers. One of the most urgent needs, says Marie-Hélene Marchand, secretary-general of the Pasteur Institute, is for books and journals. The Pasteur is currently trying to put together complete series of the major research journals for the past decade and to provide subscriptions for this year.

Another initiative, at the request of the Instituto Cantacuzino, is to administer funds it has received from abroad (notably from Belgium). Products and equipment will be ordered and paid for in France and sent to the institute, which lacks even the bare essentials for accounting.

The Cantacuzino Institute has historic links with the Pasteur and its founder, Jean Cantacuzino, was himself a Pastorian. Its present director, Auber Combiescu, grew up in France. Romanian-born researchers at the Pasteur Institute have been instrumental in reopening contacts.

Peter Coles

WORLD HEALTH ORGANIZATION

New man takes over

London

MICHAEL Merson, a US citizen, is the new head of the World Health Organization's global programme on AIDS. He replaces Jonathan Mann, who resigned in March after disagreements over policy with WHO director-general Hiroshi Nakajima (see *Nature* 344, 283, 22 March 1990).

Merson has headed WHO's diarrhoeal disease-control programme since 1984, and also took control of its acute respiratory infections programme in 1987. Addressing the 43rd World Health Assembly in Geneva after his appointment, Merson said that WHO's continued cooperation with other non-governmental organizations (NGOs) is essential in the fight against AIDS. Nakajima is believed to have opposed Mann's moves to increase links between the AIDS programme and other NGOs. But Merson's position on the distribution of drugs in developing countries, understood to be another source of disagreement between Mann and Nakajima, is less clear.

Merson's immediate concern is the AIDS programme's budget, planned for $100 million in 1991. But only $70 million has been set aside so far. Merson expressed "great concern" if this shortfall cannot be made up, and warned against "complacency" over the global AIDS pandemic.

Peter Aldhous

IN VITRO FERTILIZATION

Children 'normal'

London

CHILDREN conceived by *in vitro* fertilization (IVF) seem no more likely to suffer congenital abnormalities than the general population, a Medical Research Council working party has concluded. But the infant mortality and still-birth rate in IVF children is twice the UK average because of the high proportion of multiple births.

The survey looked at 1,267 pregnancies in the United Kingdom from 1978 to 1987, conceived by IVF or gamete intrafallopian transfer (where sperm and eggs are deposited in the fallopian tubes), which resulted in 1,581 live and still-births. Its publication in last week's *British Medical Journal* is timely, because the Human Fertilization and Embryology Bill, currently passing through parliament, will set up a Statutory Licensing Authority to regulate IVF.

The present Interim Licensing Authority guidelines recommend that three IVF embryos should be placed into the uterus to ensure a reasonable success rate. But this increases the rate of multiple births. In the opinion of Brian Lieberman, from St Mary's Hospital in Manchester, improved preservation of frozen embryos over the next two or three years should mean that multiple births will be common: if spare embryos are preserved, then there will be less initial pressure to obtain successful implantation.

Peter Aldhous

EASTERN EUROPE

A friend from Wall Street

Prague

George Soros, an expatriate Hungarian who made millions on Wall Street, is to put up $25 million as seed money for a new English-language university in Prague through his New-York-based 'Open Society Fund'. If delicate negotiations with the interim Czechoslovak government can be completed by the end of this month the university, the first of its kind in Central Europe, may open its doors as early as 1991.

Soros, who is 59, has supported cultural and educational causes in Hungary since 1984 by providing nearly $3 million a year through the 'Soros Foundation — Hungary', an offshoot of the Open Society Fund. Before the recent ousting of the Communist government, the foundation was known in Budapest as the 'alternative ministry of culture'. The new 'Central European University,' will offer post-graduate courses of up to three years and "support the transition to a market economy", according to physicist Petr Pajas, director of the project in Prague. Although the initial focus will be on the social sciences — a field that was especially deformed by Communist governments according to Pajas — the university may eventually offer courses in natural sciences such as ecology and 'evolution theory' which were also neglected.

The lone remaining obstacle to the project is finding a building in Prague suitable for intensive use as a teaching and research centre. Pajas hopes that the negotiations with the government will proceed smoothly so that the tasks of finding faculty and supplies can begin next month.

The $25 million from Soros will be used to attract faculty members, to set up special short courses in the 1990–91 academic year and to lay the groundwork for the full-scale start of the university in the fall of 1991. Fifty students will be taken in at first but Pajas expects numbers to rise to 300. Intensive training in English will be offered at least in the first two years.

Although the building will probably come from the government, Pajas stresses that the goal is "to keep the university as independent of the state as possible". The university will seek private donations for computers, books and other supplies.

An additional site has been chosen at Bratislava, the capital of Slovakia, just east of Vienna. Although the main campus will be in Prague, the full university may eventually feature satellite campuses in Budapest — where Soros has already set up an international management school with Western professors — Vienna and Graz. **Steven Dickman**

CZECHOSLOVAK ENVIRONMENT

Self-help plan

London

The communist legacy of poor training and research management in the environmental sciences compounds Czechslovakia's pollution problems, a Czechoslovak delegation revealed last week. The delegation, led by Zdenek Kukal, research director of the national geological survey, was in London to formulate proposals for support from Britain's 'Know How Fund' for Czechoslovakia. "This is about helping the Czechs to help themselves", says Professor Mike Boulter, from the Polytechnic of East London, who organized the meeting.

Karel Urban, from the Ministry of Culture and Nature Protection, explained that ecology had been viewed as an 'anti-socialist' subject. Data on Czechoslovakia's environmental crisis have now been released, and the powerful green lobby is demanding urgent action. But the newly formed environment ministry is financially stretched, lacking even a central headquarters building.

Czechoslovakia has enough graduates in the basic biological sciences, Urban said, but there is a shortage of environmental scientists. Other delegates berated the traditional bias against applied environmental research, and argued that a new system of research management is needed. "We are amateurs in managing research", Kukal said, and suggested that a group of Czechoslovaks should visit Britain to learn from the British system.

Viktor Hoschl, a self-labelled "child of the revolution" and geophysicist with Geoindustria (a state-owned company), said that exchange of information with the West is needed to help Czechoslovakia deal with its accumulating nuclear waste. Under communist control, waste was sent to the Soviet Union. Peter Aldhous

CZECHOSLOVAK ACADEMY

New laws for old

Prague

Two pieces of legislation of vital interest to the scientific community are among the 63 new laws passed by the Czechoslovak parliament since its reconstruction at the beginning of the year. But there is general anxiety about one of these bills, the law on the universities and higher education, rushed through before the end of the present session.

Essentially, the new law gives more autonomy to university rectors and deans, allows students to participate in university government and opens teaching posts to competition. The fear is that the slapdash way in which the law was drafted may conceal snags that defeat aspirations for the future.

By contrast, the amendment to the law on the Czechoslovak Academy of Sciences, was passed without excitement. The amendments give the elected representatives of academy institutes rights equivalent to those of members of the academy. The new law also abolishes the requirement that academy scientists should compete for their jobs every five years, which had been used for blackmail by the previous regime. However, membership may now be terminated on the grounds of unethical conduct or in cases of members appointed for "non-scientific" reasons.

The legislation also legitimizes the way in which the academy has operated since last December, since when a panel of seven elected scientists has shadowed the decisions of the formal praesidium, exercising a veto on matters with which it disagreed. Now it should be possible for those in charge to turn from criticism of the old order to finding a more logical and less discriminatory way of conducting research. **Veronika Maxova**

SOUTH AFRICAN UNIVERSITIES

Lesotho academic excluded

Cape Town

The decision to refuse a South African residence permit to Professor Njabulo Ndebele, a citizen of Lesotho who was chosen by the University of the Western Cape (UWC) to be its second vice-rector (see *Nature* **344**, 581; 12 April 1990) was made at cabinet level, according to a statement released by the Minister of Home Affairs and National Education, Gene Louw, after a meeting with UWC rector Jakes Gerwal and vice-rector Jaap Durand on 17 April.

Louw said that Ndebele, an exiled South African, was placed on the government's list of persons to whom visas should not be granted several years ago, and that it was "not customary" for the government to provide reasons why individuals' names appeared on the list. Louw claimed that government "was accommodating within all reasonable limits" about requests to grant visas to listed people, but "could not be expected to simply submit to all requests from parties who do not possess all the facts". UWC's council has asked for a meeting with President de Klerk to discuss the case.

Although Louw was dealing with this issue in his capacity as Minister for Home Affairs, his action bodes ill for Louw's future relations with the universities as Minister for National Education, his second cabinet portfolio. Louw took over the post last month from Dr Gerrit Viljoen, who is concentrating on his responsibilities as Minister for Constitutional Development and chief negotiator with the African National Congress.

Michael Cherry

JAPAN'S ANTI-NUCLEAR MOVEMENT

Yen melts down opposition

Rokkasho

THE opening of Japan's first commercial facility for the enrichment and reprocessing of nuclear fuel and storage of nuclear waste is destined to go ahead despite local and nationwide opposition.

Last week, local government officials, representatives of the facility and fishermen opposed to the nuclear complex in Rokkasho village on the northern tip of the main island of Japan made it clear to visiting members of the foreign press that the huge amounts of money brought in by the project have effectively quashed almost all local opposition.

The Rokkasho facility, which will cost well over ¥1 million million ($6,500 million) when completed in the late 1990s, is central to Japanese government plans to gain greater independence from foreign suppliers of nuclear fuel. At present nearly all uranium used in Japan's nuclear power plants is of US origin and Japan's spent fuel has to be sent to the United Kingdom and France for reprocessing.

The first facility at Rokkasho, scheduled to open next year, will be a ¥180,000 million plant for the enrichment of uranium. The plant, to be run by Japan Nuclear Fuel Industries (JNFI), a consortium of ten electric power companies, is already 60 per cent complete, according to JNFI officials, and when it reaches full capacity in 2000 the plant will be capable of producing about 20 per cent of Japan's enriched uranium needs.

A low-level waste storage facility also owned by JNFI is expected to open in 1992 at a cost of over ¥100,000 million. With enough space for three million 200-litre drums of waste the facility will be capable of absorbing all low-level waste from Japan's power stations well into the next century. The drums will be buried 12 metres underground, encased in concrete, and monitored for leakage for the next 300 years.

Facilities for reprocessing spent fuel will be built by another consortium of over 100 companies, Japan Nuclear Fuel Service (JNFS), at a cost of ¥840,000 million. JNFS officials hope to start accepting spent fuel from Japanese power stations in 1994 and to begin reprocessing it into uranium and plutonium in 1997 after the fuel has 'cooled down'.

At full capacity, the plant will be able to reprocess the spent fuel from about 30 of the 37 nuclear power reactors currently in operation in Japan. The remaining spent fuel will continue to be sent to the United Kingdom and France for reprocessing.

Japanese high-level waste from the reprocessing plants in Europe will be stored 'temporarily' at Rokkasho for 20 to 30 years from 1993 onwards, according to JNFS officials.

Since the Chernobyl accident in 1986, the Rokkasho facility has become the focal point of a growing nationwide anti-nuclear power movement. And in December the incumbent village chief in Rokkasho, a strong supporter of the facility, was ousted in the village election by Hiroshi Tsuchida who campaigned for a freeze until the safety of the facility is confirmed.

Last week, Tsuchida, a former supporter of the project, said that he is personally convinced of the plant's safety and once he feels that about 75 per cent of the approximately 12,000 villagers are also convinced he will allow commercial operations to begin (the village chief must sign a safety agreement with JNFS and JNFI before commercial operations can start). Tsuchida estimates that only a few hundred locals are vehemently opposed to the plants and he and fellow village assemblyman Saburo Hashimoto, a supporter of the project, say that JNFS and JNFI should carry out more public relations campaigns to win over the large number of people taking a 'neutral' stance. In line with such calls, the two companies plan to open a new ¥3,000 million ($2 million) public relations centre at Rokkasho next year.

Members of an association of 30 fishermen in the nearby village of Tomari who are opposed to the facility claim that over 50 per cent of Rokkasho villagers are against the project and that many people were deceived by Tsuchida's call for a freeze. But the association says that people cannot openly express opposition because they would be turned away from part-time jobs at farms and the construction site for the facility by village officials who are promoting the project. During the construction peak in the next few years, thousands of locals will be temporarily employed to construct the plants.

And in the Rokkasho area, where men often have to go to Tokyo to find employment as part-time construction labourers, the incentive to refrain from criticizing the project is strong, the Tomari fishermen say. But construction jobs are not the only incentive. Hashimoto says that under a Japanese law designed to promote nuclear power, the Ministry of International Trade and Industry (MITI) will provide Rokkasho with ¥18,000 million ($120 million) in subsidies over a ten-year period. This amounts to about $10,000 for every man, woman and child in the village.

The money, which is over four times the village's annual budget, will be used for 96 new village projects including a fish farm, facilities for manufacture of fertilizers, roads, radar facilities for fishermen, a large refrigerated warehouse for storing vegetables, a sports amenity centre and a ski resort. Another ¥18,000 million will be given to several villages surrounding Rokkasho, Hashimoto says.

The fishermen's association has filed suit in the Tokyo district court against former prime minister Noboru Takeshita for approving construction of the uranium enrichment facility. The land at Rokkasho was bought from local people by the government-backed Mustu Ogawara Corporation during the 1960s, on the understanding that it would be used for industrial development. But the fishermen do not expect success. They are pinning their last hopes on support from a nationwide network of anti-nuclear activists and on the success of an anti-nuclear candidate in the elections for governor of Aomori Prefecture to be held next February.

David Swinbanks

ARCHAEOLOGY

US cracks down on Peru loot

Washington

AFTER a three-year investigation into the desecration and looting of the richest tomb ever excavated in the Western Hemisphere, the US Customs Service has announced a ban on the importation of all archaeological artefacts from the Sipán region of Peru. The US Customs Service said it would seize any artefact that was not accompanied by documentation from the Peruvian government certifying that it had left the country legally. "We are appalled by the desecration of the Sipán Region", said Customs Deputy Commissioner Michael Lane, "but we will end this crime." The discovery in 1987 of the graves of the 'Lords of Sipán', as the site is known, led to the 'Peruvian Gold Rush', a frenzy of looting that robbed the tombs of thousands of priceless pre-Columbian objects. Many of the items eventually made their way to the United States, where they were bought by eager collectors.

...and we've arrested a Dr. Indiana Jones..
U.S. CUSTOMS
BIRCH

In 1988, armed US customs agents searching for illegal Peruvian artefacts raided a group of homes and businesses in Southern California. Among the collectors caught in the raid was physics Nobel laureate Murray Gell-Mann, who had invested tens of thousands of dollars in Sipán art and relics. Gell-Mann then worked with the investigators, and returned several dozen artefacts to Peru.

G. Christopher Anderson

Charting the decline

SIR—The letter from J.F. Lamb[1] has prompted us to examine some data extracted from a recently published study[2]. Table 1 shows country-by-country mean citation rates of papers published in *Nature* during 1981–85 as related to the mean citation rate of all papers published in *Nature* in the same period. All countries publishing more than ten papers are shown.

TABLE 1 Relative Citation Rate (RCR) of papers in *Nature*

Country	RCR	Country	RCR
Sweden	1.92	Poland	0.70
Switzerland	1.78	Italy	0.67
Japan	1.46	Austria	0.67
USA	1.18	Norway	0.64
West Germany	1.17	Canada	0.62
Denmark	1.07	USSR	0.52
Israel	1.05	Australia	0.46
The Netherlands	1.01	Spain	0.46
France	0.92	Brazil	0.41
Belgium	0.79	New Zealand	0.41
United Kingdom	**0.77**	Ireland	0.35
Hungary	0.75	South Africa	0.23
Finland	0.70	India	0.14

The relative citation rate (RCR) has a value greater than 1.00 when the papers of the country in question are cited above the journal's average and conversely. *Nature* published 8,043 papers in 1981–85 with an average citation rate of 16.63.

As can be seen, Sweden heads the ranking, followed by Switzerland, Japan, the United States and West Germany. The United Kingdom is ranked eleventh. This could be considered to be in line with the reports of Lamb and others on the decline of British science[3,4] although other studies did not find any statistically significant decline in the indicators of British science during the 1980s[5-8]. For that reason, we consider the whole problem of the decline of British science is still open to discussion — leaving aside questions such as what is meant by decline[9] and whether whatever it is is correlated with the decline in funding, for example.

Accordingly, we have repeated the same exercise with another 'high quality' periodical, the *Biochemical Journal*.

TABLE 2 Relative Citation Rate (RCR) of papers in the *Biochemical Journal*

Country	RCR	Country	RCR
United Kingdom	**1.22**	Norway	0.80
Sweden	1.21	Canada	0.79
Switzerland	1.16	France	0.79
Belgium	1.12	Spain	0.79
Denmark	0.92	Austria	0.79
The Netherlands	0.92	Hungary	0.74
South Africa	0.90	Ireland	0.67
West Germany	0.89	Finland	0.66
USA	0.89	Poland	0.64
Australia	0.87	Argentina	0.63
Israel	0.82	USSR	0.49
Japan	0.81	India	0.41
New Zealand	0.80	Chile	0.25

Table 2 presents the results, covering 4,475 papers published in 1981–85, with an average citation rate per paper of 6.90. As will be seen, the United Kingdom is ranked in first position.

Our sole purpose here is to suggest that a counting such as that of Lamb is not by itself a sufficient measure of decline.

Bibliometric methods are not the most serviceable tools unequivocally to prove such a decline during the 1980s. A more realistic approach would show[2] that British science, like science in other countries, is in decline in some fields and on an upward move in others.

Moreover, in any population of papers, the distribution according to quality (citation rate is but a proxy) is highly skewed. Our investigations[2] indicate that probably the most important factor in improving scientific performance of a country is finding a way to raise the quality and not quantity of the publications by influencing the skew distribution to have a low quality 'tail' as short as possible.

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1. Lamb, J.F. *Nature* **343**, 404 (1990).
2. Schubert, A., Glänzel & W. Braun, T. *Scientometrics* **16**, 3 (1989).
3. Irvine, J., Martin, B., Peanock, T. & Turner, R. *Nature* **316**, 587 (1985).
4. Martin, B.R., Irvine, J., Narin, F. & Sterritt, C. *Nature* **330**, 123 (1987).
5. Leydesdorff, L. *Science and Public Policy* **15**, 270 (1988).
6. Nederhof, J. *Scientometrics* **14**, 475 (1988).
7. Braun, T., Glänzel, W. & Schubert, A. *Scientometrics* **15**, 165 (1989).
8. Braun, T., Glänzel, W. & Schubert, A. *The Evaluation of Scientific Research*, Ciba Foundation Conference, p. 32 (Wiley, Chichester, 1989).
9. Nye, M.J. *ISIS*, **75**, 697 (1984).
10. Babbage, Ch. *Nature* **340**, 499 (1989).

Growing dissent

SIR—The debate between advocates of sustainable economics and pro-growth economics is more complicated than implied by the recent leading article, "Doctrinal fallacies of stewardship" (*Nature* **344**, 179; 1990).

(1) The assumption that funding for scientific research and development entails economic growth cannot be cogently defended. While we agree that scientific enquiry is at present underfunded, both in Britain and elsewhere, the explanation cannot be attributed to any single factor.

(2) Clearly the term 'economic growth' has a ceremonial value, while 'no-growth lobby' has a pejorative ring. Yet even radical 'greens' are in favour of 'economic growth': the issue is what kind of growth. The article begs several questions. Just what, for example, do the national income accounts at present measure?

(3) While the article seems relatively sanguine about the future of European civilization, there is a remarkable inconsistency inherent in the assertion that Africa, "even before AIDS, seemed destined to be the first continent to be lost to human habitation through wayward mismanagement". Even assuming, out of ethnocentric arrogance if nothing else, that Africans bring the house down on their own heads, an ecological viewpoint forces recognition that our own self-interest is at stake. Africa is part of the global ecosystem.

(4) No responsible scientist claims to be able to predict the future (unless all other conditions are equal), yet the essay skates perilously close to prophecy on more than one front. Chaos theory, if it means anything *vis-à-vis* the environmental policy debate, implies that small and seemingly insignificant variables can have major unpredicted effects. Clearly, some kinds of economic growth could have unpredicted and disastrous ecological effects. Furthermore, even assuming adequate funding for basic science research, there is no guarantee that the knowledge necessary to sustain civilization as we know it will be forthcoming.

(5) Finally, the article ignores a host of so-called science–society issues that such luminaries as Albert Einstein and Erwin Schrödinger have raised.

Rather than being the last word, we hope that the leading article serves the function of opening up and extending the conversation. Perhaps the wide range of articles dealing with various facets of ecology, environment and human impacts that have graced the pages of *Nature* are the beginning of a vital process of public deliberation informed by scientific inquiry.

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Missing woman

SIR—Sachi Sri Kantha (*Nature* **344**, 582; 1990) concludes that 21 women so far have been awarded the Nobel prize. However, at least one woman was missing. In 1909, the Swedish novelist Selma Lagerlöf was the first woman to receive the literature prize.

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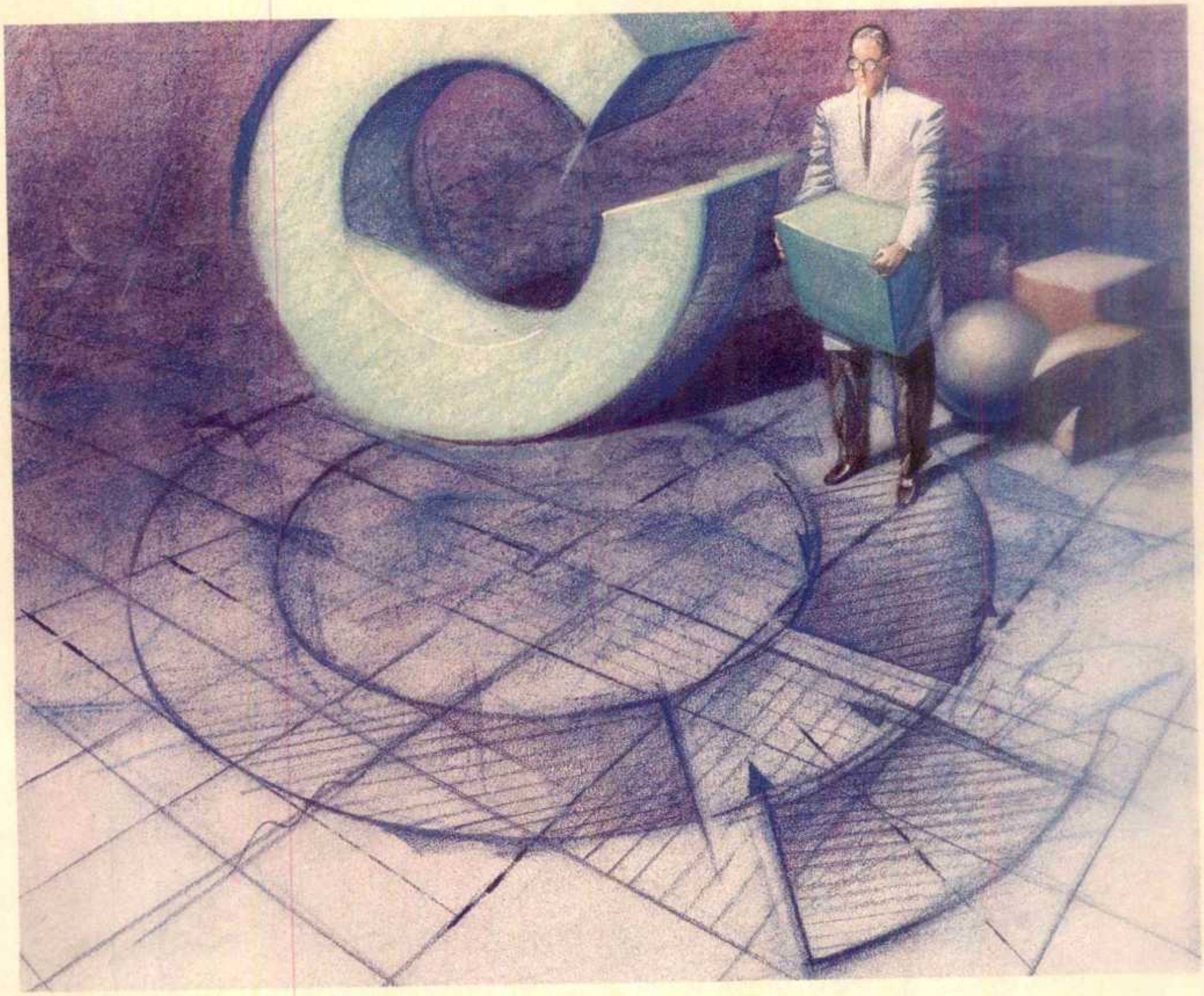

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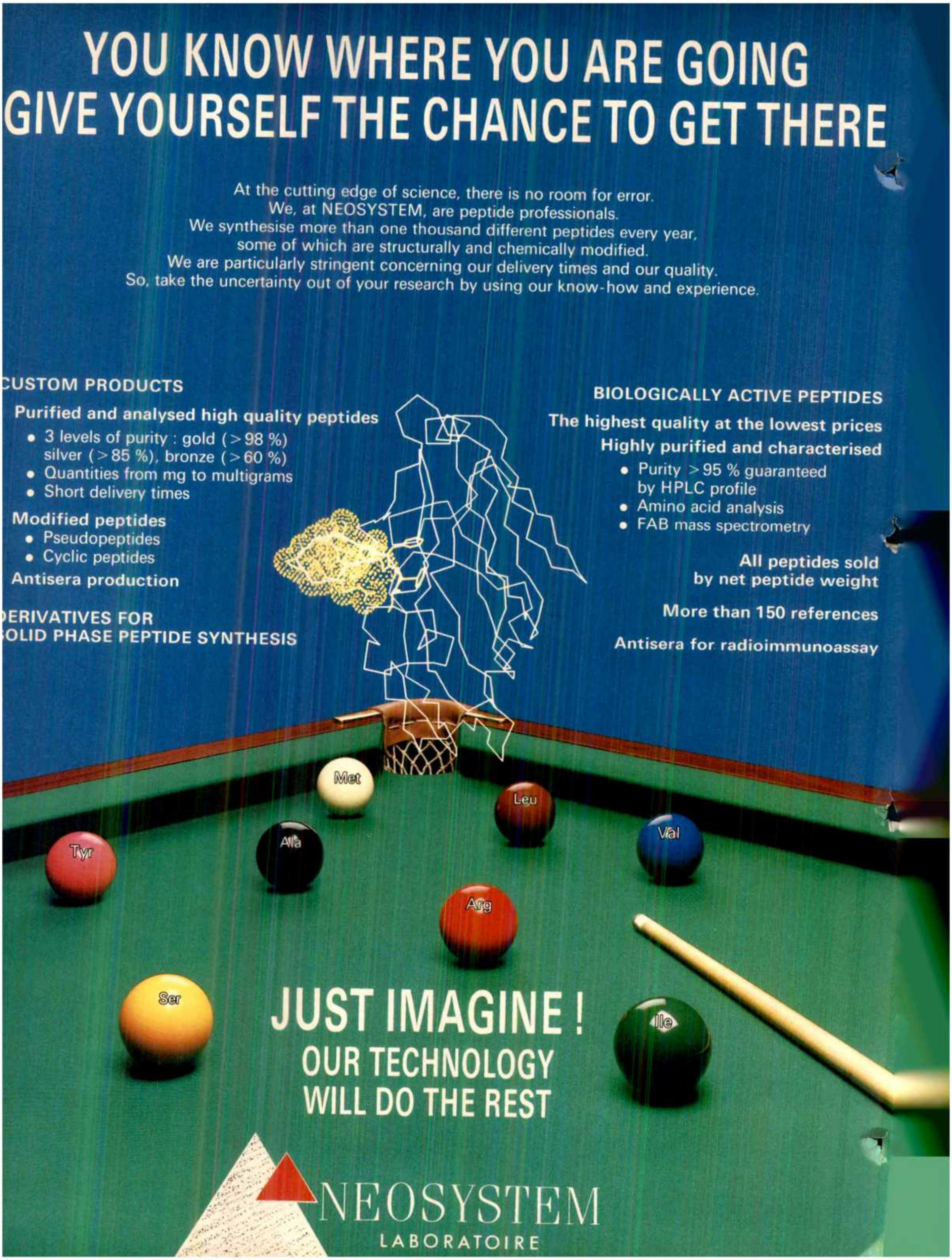

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What price the global greenhouse?

Is the global greenhouse potentially a calamity to be avoided at all costs? Or an unavoidable disaster of incalculable cost? One view is that the best greenhouse is that which, in the broadest sense, costs least.

Munich

THERE are two distinct and extreme views of the consequences of the general increase of temperature likely to be occasioned by the accumulation of carbon dioxide and other greenhouse gases in the Earth's atmosphere. One is that even the slightest departure from the present fortuitous pattern of climate will be tantamount to a calamity and had better be avoided. The other is that modern technology is now so sophisticated that even the most severe departures from the present state of affairs can be accommodated.

Both positions are, of course, mistaken. Climatic changes indistinguishable from interannual noise would be of no importance, while changes much more rapid than the timescale on which the world's stock of social capital is renewed — say, a century or so — would clearly be a great economic if not absolute embarrassment. What can be said of more modest and more tenable intermediate positions?

In this connection, one should acknowledge that there is some force in the extreme environmentalists' position that even modest change means calamity. Most calculations of the greenhouse effect yield estimates of the increased temperature caused by a doubling of atmospheric carbon dioxide, which on present trends is expected to happen about the year 2035. The modellers seem to agree that the extra temperature then or some decades later (if the oceans substantially delay events) could be as much as 4° C. Most arguments about the adequacy of the models centre on assertions that ameliorating feedback mechanisms may have been overlooked in their construction. But if nothing else is at stake, the result must simply be that the full rigours of the predicted greenhouse are delayed. That is another way of saying "In the long run, we are all dead".

It is nevertheless worth asking what will happen if the projected change of climate does occur in the next few decades. One possibility is that governments might not act energetically enough, or in time. But there is also a colder calculation to be made: on the face of things, avoidance may be the prudent course, but to what degree?

In some broad sense, this is an economic question. The cost of not avoiding rapid climatic change is that of adapting to the conditions that climatic change would bring. The cost of avoiding the greenhouse absolutely would be that of maintaining constant the atmospheric concentration of carbon dioxide and other greenhouse gases. It is unlikely that the most economical course to follow would be that of absolute avoidance. Nor is the cost of doing nothing likely to be the smallest. By these criteria, and in an ideal world in which all decisions are taken rationally, the best strategy would almost certainly be a calculated blend of avoidance and adaptation.

The obvious trouble is that such questions cannot be made even partially precise in present circumstances. It is not merely that the calculations have not been carried out, but that the basis on which they might be attempted are essentially undefined. What, for example, is the probable effect of climatic change of the kind predicted on the economic basis of city life? Increased temperatures might for example bring reduced heating costs but increased costs for air conditioning. Where would the balance lie, and would the trade-off be such that net energy consumption and thus ultimate greenhouse-gas production would be increased or decreased? Could the radical redesign of buildings change the balance and ameliorate it and, if so, to what degree? What would be the consequences of markedly higher electricity costs or restrictions on the use of internal combustion engines?

The economic basis of industrial activity is even less thoroughly explored, although some rules of thumb can be inferred from past experience. To the extent that restrictions on the release of greenhouse gases, and carbon dioxide in particular, will appear in the economy as increased energy prices, attempts to regulate the greenhouse gases will have effects not very different from the rapid increases of the price of oil in 1973 and 1979, although the opportunities for substitution (by coal, for example) will be restricted. That suggests that energy-intensive metals (such as steel and aluminium) will be at a disadvantage, while refined products of all kinds will become more expensive relative to services provided by people. But to what degree? And to what extent can novel technology provide alternatives? These questions are imponderable, but nevertheless important.

Unsurprisingly, the one field of economic activity to which some attention has been paid is that of agriculture. The explanation is not difficult to fathom. The effects of climatic change on the growth of crops is, after all, direct; increased temperature and reduced (or increased) rainfall has a direct effect on the way plants grow. So, for that matter, has the increased concentration of carbon dioxide in the atmosphere, which generally enables photosynthesis to be more rapid, but to an extent that varies from one crop to another.

One of the most elaborate studies so far of the effects of climatic change on agriculture is that reported in last week's issue by R. H. Adams *et al.* (*Nature* **345**, 219; 1990), which vividly illustrates the complexity of even the simplest studies aimed at throwing light on the way in which subsystems of the modern economy are likely to respond to climatic change. For one thing, the study is concerned only with US agriculture, which is the best understood of any, and with only three crops. For another, while dependent for its calculations on model projections of climatic changes caused by greenhouse gases, it must use a mere handful of experimental data on the increase of the rate of photosynthesis in different crop plants caused by increased carbon dioxide.

The conclusions are also complex, and in some ways unexpected. US agriculture could survive climatic change of the kind that may be caused by a doubling of carbon dioxide in the atmosphere, at least if full credit is taken for the stimulation of photosynthesis by carbon dioxide. There would even be agricultural produce left over for the strategically important export trade. But at a cost. Irrigation needs, already met only at high cost, would multiply. Food consumers would have to pay much more for food relative to other consumer prices. Just how farmers would fare is another matter: the two climate models used in the study suggest that the higher prices hurting consumers would (not surprisingly) benefit farmers, giving them a little extra income.

The authors of the study now say that they are unhappy that the last of these issues has been singled out for special attention in the general press, but is that necessary or even wise? The value of a study such as this is not to predict the future, but rather to provide an understanding of a complex economic field that is rich enough to allow for rational planning, preferably before the reality of the greenhouse is palpable. Does it matter that, among the majority of losers, some may be better off?

John Maddox

HIV-1 ORIGINS

A finger on the missing link

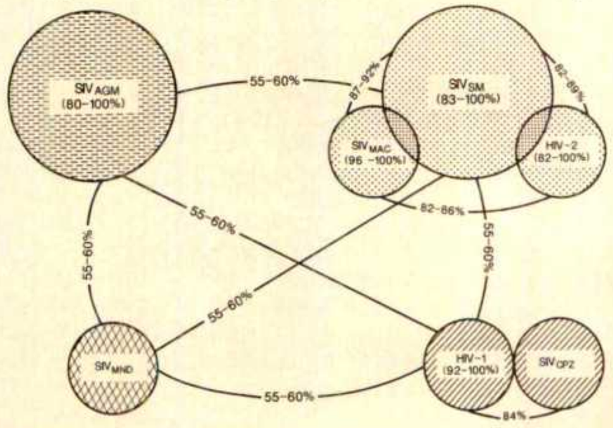
Ronald C. Desrosiers

PREVIOUSLY unrecognized and apparently new, AIDS burst onto the scene in the 1970s and 1980s in the United States, central Africa, Europe and elsewhere. But where did the causative agent, a human lentivirus called HIV-1, come from? Although the report by Huet *et al.* on page 356 of this issue[1] provides no final solution to the mystery, the authors have uncovered the most significant clues to date. Their genetic sequence of a lentivirus isolate from a chimpanzee shows it to be much more closely related to HIV-1 than any previous primate lentivirus isolates, yet it is outside the range of variation of known HIV-1 isolates.

One of two possibilities seems likely for the origin of HIV-1. First, the virus may have always been present in the human population, in one form or another, but may have gone unrecognized due either to an extremely low prevalence or its confinement to isolated populations. Some features of modern society, for example re-use of syringes and hypodermic needles, changes in migration patterns with extensive worldwide travel, and sexually promiscuous activities, could have greatly facilitated its spread in recent history. Second, HIV-1 may have entered the human population by transmission of a virus from another species relatively recently in history. Increasing prevalence and high mortality would then be consequences of infection of the new host.

Related lentiviruses have now been isolated from sheep, goats, horses, cattle, cats, monkeys and humans. The simian immunodeficiency viruses, or SIVs, are nonhuman primate lentiviruses that are the closest known relatives of the HIVs. SIVs have previously been isolated from macaques (SIV_{MAC}), sooty mangabeys (SIV_{SM}), African green monkeys (SIV_{AGM}) and mandrills (SIV_{MND}) (refs 2–6). Mangabeys, green monkeys and mandrills are African Old World primates, whereas macaques are Asian Old World primates. These four SIVs fall into three discrete groups based on genetic sequence analysis, with SIV_{MAC} and SIV_{SM} forming a single genetic group[7-10]. Macaques are apparently not infected with SIV in their native habitat[5,11], so it seems likely that some individual macaques became infected with SIV_{SM} from sooty mangabeys while in captivity at US Regional Primate Research Centers[9,12]. Genetic evidence as well as geographical considerations provide circumstantial evidence that sooty mangabeys, or some close relative, may also have been a source of cross-species infection of humans for the origin of HIV-2 in West Africa[9,12]. HIV-2 is no more different from SIV_{SM} at the sequence level than individual SIV_{SM} isolates are different from each other (refs 9, 12; Y. Li *et al.*, unpublished observations). Furthermore, the natural habitat of sooty mangabeys is the coastal forest regions of West Africa, the same region where HIV-2 is endemic. Thus, this type of molecular detective work has already lent support to the cross-species transmission theory for the origin of HIV-2. But because SIV_{MAC}, SIV_{SM}, SIV_{AGM} and SIV_{MND} are only distant relatives of HIV-1, the source of worldwide human infection with HIV-1 has remained a mystery.

The lentivirus isolate sequenced by Huet *et al.*[1] was derived from a captive chimpanzee in Gabon[13], and termed SIV_{CPZ}. It is the only lentivirus isolate other than HIV-1 that has a genomic organization that includes the genes *gag*, *pol*, *vif*, *vpr*, *tat*, *rev*, *vpu*, *env* and *nef*. Furthermore, it is much more closely related to HIV-1 at the sequence level than any of the other lentiviruses. For example, amino-acid identities in the *gag* and *pol* gene products are 75 and 84 per cent (see figure). By comparison, the other SIVs have only 55–60 per cent amino-acid identity in these products when compared with HIV-1. The other animal lentiviruses have even less (25–30 per cent). Nonetheless, the chimpanzee lentivirus sequence is outside the range of variation of known HIV-1 isolates; even the most divergent isolates of HIV-1 have about 92 per cent positional identity in these gene products. So the chimpanzee lentivirus isolate may be a form of 'missing link' between the African Old World primate SIVs and HIV-1.



Primate lentivirus taxonomy. Percentages in the lines are amino-acid identities in the *pol* gene product; those in parentheses are the ranges of identities in the product in isolates of each virus.

Ardea

The chimpanzee — a new subject in the hunt for the origins of HIV-1.

These observations do not, however, allow discrimination between the theories for the origin of HIV-1. The data that have been obtained so far are conceivably consistent with both hypotheses. For many virus groupings, related viruses can be found throughout the animal kingdom. Their relatedness to the human virus generally reflects the evolutionary distance of the host species. For example, viruses related to herpes simplex virus (HSV) of humans can be found all through the animal kingdom and their relatedness to HSV in general reflects the evolutionary distance of the host species[14,15]. Apparently, an ancestor alpha herpes virus was present very early in evolutionary history and the viruses diverged in concert with their host species. The gradient of sequence relatedness from the ungulate lentiviruses, through SIV_{AGM}, SIV_{SM} and

SIV_{MND}, to SIV_{CPZ} and HIV-1, is consistent with such coevolution. However, if SIV_{CPZ} is part of a large, diverse group similar to SIV_{AGM} (ref. 16) and SIV_{SM} (Y. Li *et al.* unpublished observations), then HIV-1 could also have been derived by cross-species transmission from an individual member of the SIV_{CPZ} group.

Many questions still surround the SIV_{CPZ} isolate. Fortunately, they are amenable to experimental investigation and their resolution will allow us to decide between the competing theories for the origin of HIV-1. Is the virus really a natural infectious agent of chimpanzees? Hundreds of captive chimpanzees have been examined previously and none were seropositive except those intentionally inoculated with HIV-1 or with human materials[17]. The chimpanzee from which SIV_{CPZ} was isolated was one of only two seropositives among the 83 tested in the captive Gabon colony[13]. In contrast, 20–50 per cent of African green monkeys in their native habitat are naturally infected with their own SIV. Is there any chance that this chimpanzee became infected from a human source? Chimpanzees occasionally have been observed to eat monkeys in the wild — is it possible that this chimpanzee became infected from some other nonhuman primate species?

If the virus is indeed a natural infectious agent of chimpanzees, it will be important to determine the prevalence and demographics of infection and to document the extent of genetic diversity among an array of isolates. The status of other great apes will also need to be investigated. If SIV_{CPZ} turns out not to be a natural infectious agent of chimpanzees, it will be important to find the source of infection, particularly if it is some other nonhuman primate. What is clear is that the proliferation of SIV isolations has created a fascinating molecular–genetic game to unravel the origins of the HIVs. ☐

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1. Huet, T. *et al. Nature* **345**, 356–359 (1990).
2. Daniel, M. D. *et al. Science* **228**, 1201–1204 (1985).
3. Murphey-Corb, M. *et al. Nature* **321**, 435–437 (1986).
4. Fultz, P. N. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 5286–5290 (1986).
5. Ohta, Y. *et al. Int. J. Cancer* **41**, 115–122 (1987).
6. Tsujimoto, H. *et al. J. Virol.* **62**, 4044–4050 (1988).
7. Chakrabarti, L. *et al. Nature* **328**, 543–547 (1987).
8. Fukasawa, M. *et al. Nature* **333**, 457–461 (1988).
9. Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. *Nature* **339**, 389–392 (1989).
10. Tsujimoto, H. *et al. Nature* **341**, 539–541 (1989).
11. Daniel, M. D. *et al. Int. J. Cancer* **41**, 601–608 (1988).
12. Desrosiers, R. C., Daniel, M. D. & Li, Y. *AIDS Res. Hum. Retroviruses* **5**, 465–473 (1989).
13. Peeters, M. *et al. AIDS* **3**, 625–630 (1989).
14. Roizman, B. *et al. Intervirology* **16**, 201–217 (1981).
15. Desrosiers, R. C. & Falk, L. A. Jr *J. gen. Virol.* **56**, 119–130 (1981).
16. Li, Y., Naidu, Y. M., Daniel, M. D. & Desrosiers, R. C. *J. Virol.* **63**, 1800–1802 (1989).
17. Gilden, R. V. *et al. Lancet* **I**, 678–679 (1986).

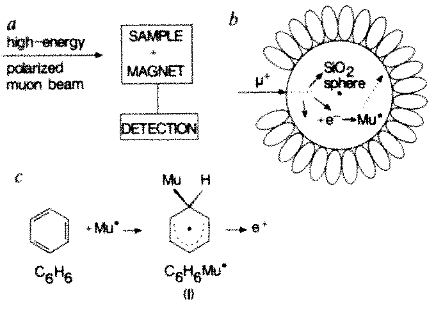
PHYSICAL CHEMISTRY

Snapshooting surface radicals

Martyn C. R. Symons

IN heterogeneous catalysis, surface-adsorbed molecules are generally the principal reactive species. But the intermediates of such chemical transformations (free radicals, for example) are notoriously difficult to detect because they exist for only a short length of time, and their concentration at any instant therefore tends to be very low. Reid *et al.* now report on page 328[1] a new method for studying possible radical intermediates on solid surfaces. These experiments involve directing a beam of very-high-energy positive muons (μ^+) through the sample — in this case, silica spheres with a monolayer of benzene ($SiO_2-C_6H_6$) — and studying its chemical effects by detecting the positrons that are formed by muon decay about 2×10^{-6} s after the exposure. The radical detected was the muonated cyclohexadienyl radical — as expected, because it is also formed when pure benzene is exposed to muons. Reid *et al.*'s exotic technique allows this radical to be detected despite its short lifetime and its very low concentration. Also, the variations of line-width of the muon spectrum with temperature give information about the motion of these radicals.

Positive muons can be thought of as light protons, the mass being about one-ninth that of the proton. From a chemist's point of view, they are a fortunate product of some of the huge and costly experiments indulged in by physicists in which elementary particles are accelerated and induced to interact with each other. When the muon beam impinges on a collection of molecules, radiation chemistry occurs in the same way as, for example, it does on exposure to high-energy protons, electrons or γ-rays (see figure). In the $SiO_2-C_6H_6$ experiment, high-energy μ^+ particles will penetrate the system, the most probable reaction resulting in the formation of muonium atoms Mu (this is the equivalent of a hydrogen atom), by electron capture. Of course, other chemical processes take place, but these are not seen by the detection system, which is concerned only with the muons and the associated decay positrons.



a, A muon beam is isolated from the accelerator and impinges on the sample which is housed between the poles of an electromagnet and the positron detection plates. *b*, Sketch of an individual silica grain which captures a muon to give a muonium atom (Mu). Damage caused by the beam is not detected by this technique. The Mu migrates to the surface and reacts with a benzene molecule to give a cyclohexadienyl radical, as in *c*, which then emits a positron that is detected by the very sensitive detection system.

In the absence of benzene, normal Mu atoms are detected in the silica grains — their properties correspond exactly to those of hydrogen atoms trapped in silica. For the small size of silica grains used, there is a high probability that a muonium atom generated in the grain can migrate to the surface and react with benzene in the limited time available. Thus the benzene can 'harvest' muonium atoms.

It can be deduced that the $C_6H_6Mu^•$ radicals are not free to rotate but that they migrate around the silica spheres in an unrestricted manner. This averages out the anisotropies of the muon–electron–nuclear interactions almost as effectively as free rotation.

How do these results compare with those obtained for benzene molecules using techniques such as infrared or NMR spectroscopy? Extensive studies[2,3] have shown that these molecules are equally free to migrate but not to rotate freely, the rates deduced in this way being comparable with those for $C_6H_6Mu^•$. In hindsight, it is not surprising that this should be the case, because the mass difference is negligible, and there is no specific mechanism for tying the radicals to surface groups on the spheres. Hence they are carried around with the benzene molecules.

Muon spin resonance can be most readily compared with electron spin resonance (ESR) spectroscopy[4]. The main difference between the two techniques is that the first is concerned with coupling to the muon nucleus in radicals whereas the second looks at electrons in radicals. In most cases essentially the same species are detected, and it is the unique isotope effects that are of primary interest. There are, however, some areas in which muon studies are clearly superior to ESR studies. One is in the detection of gas-phase radicals, and another is in the study of various solids, in particular industrially important materials such as crystalline and amorphous silicon, germanium and III–V (such as gallium arsenide) and II–VI (such as cadmium sulphide) compounds. Normal ESR methods are very limited in their ability to detect gas-phase radicals[5] (mainly because of rotational coupling and consequent line broadening), whereas muonated radicals can be easily detected in the gas phase. Muonated centres have been detected whose hydrogen-atom analogues have never been detected by ESR spectroscopy[6,7]. Moreover, because of their greater sensitivity, muons can be used to study the kinetics of surface reactions. Perhaps surface studies such as that described by Reid *et al.* will prove to be yet another area in which the use of muons is advantageous, although it will never be a popular method because of the extreme difficulties in obtaining muon beam time. And it must be borne in mind that the muon technique is quite limited in the types of radicals that can be detected, in contrast with ESR spectroscopy, which is also a powerful tool for studying radicals on surfaces. It seems likely, however, that those who do have access to muons will be inspired by the results of Reid *et al.* to try to probe surface reactions of industrial importance, particularly from a kinetic viewpoint. ☐

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1. Reid, I. D., Azuma, T. & Roduner, E. *Nature* **345**, 328–330 (1990).
2. Kiselev, A.V. & Lygin, V.I. *Infrared Spectra of Surface Compounds* (Wiley, New York, 1975).
3. Boddenberg, B., Haul, R. & Opperman, G. *Naturwiss.* **56**, 636–640 (1969).
4. Cox, S.F.J. & Symons, M.C.R. *Hyperfine Interactions* **32**, 689–706 (1986).
5. Carrington, A. *Microwave Spectroscopy of Free Radicals* (Academic, London, 1974).
6. Patterson, B.D. *Muons and Pions in Materials Research* (eds Chappert, J. & Grynszpan, R.I.) ch.10 (North Holland, Amsterdam, 1984).
7. Cox, S.F.J. & Symons, M.C.R. *Chem. Phys. Lett.* **126**, 516–525 (1986).

EARTHQUAKES

Seismic cycle not so simple

Max Wyss

UNTIL now it has seemed reasonable to model the seismic cycle of great earthquakes along plate boundaries as follows: at the time of a great earthquake a rupture with approximately uniform slip releases most of the available energy throughout the extent of the rupture outlined by the aftershocks. Then, plate motions accumulate strain energy at an approximately constant rate over the recurrence period (thirty to several hundred years, depending on the location), until the strain level at which rupture occurs is again reached. At that time a great earthquake re-ruptures the same plate boundary segment. This process would result in a seismic cycle of approximately constant recurrence time, because the loading rate and rupture dimensions are constant. The rupture lengths may repeatedly be the same because some geometric tectonic features may define segments of the plate boundary that will rupture in one earthquake. By showing that earthquakes that re-rupture a particular segment of plate boundaries are not similar, Thatcher[1,2] (first in *Nature* and now in full in the *Journal of Geophysical Research*) has deprived seismologists of part of their favourite model for the generation of great earthquakes.

In cases that conform closely to this model, one speaks of a characteristic earthquake[3]. There are, however, also sequences of earthquakes in nearly the same location with regular recurrence times but significantly different magnitudes[4]. Thatcher points out that the simple recurrence model is based partly on the incorrect assumption that the slip is evenly distributed over the rupture area, when in reality most of the energy release comes from a fraction of the rupture area, where the slip is large. He also shows that characteristic earthquakes are the exception rather than the rule along the circum-Pacific plate boundaries. Segments that have historically ruptured in one great earthquake often re-rupture in two or three smaller events, a few years apart. But did these subsequent ruptures really achieve the same strain release as the large one?

As Thatcher observes, the sum of the seismic moment release in several smaller events is much smaller (in the best documented case, 80 per cent less) than that of a single large event. Thus, one cannot think of the two types of rupture as equivalent, even though large events that are multiple ruptures might be interpreted as a sequence of smaller earthquakes occurring within minutes rather than within decades. The slip in the smaller earthquakes, which make up one part of the cycle, may be as little as 20 per cent of the slip in the previous cycle (the large single event), or 40 per cent over half the width of the plate boundary. One might hesitate to call something a 'seismic cycle' which is defined by subsequent episodes of energy release of 100 per cent and 20 per cent. It would be more correct to argue that in the second instance nothing has happened (no energy release) to a first approximation, than to take the view that the same cyclic event (100 per cent release) has happened again.

Thatcher retains the idea of a seismic cycle because the entire plate boundary segment is ruptured in great earthquakes each time, and the intervals remain the same. But do they? Thatcher argues that they do. But this idea may also have to be modified when developing a more complicated model. In the several cases for which Thatcher gives more than one recurrence time (at least three repeated ruptures) the differences between the shortest and longest recurrence interval is on average 67 per cent of the shortest interval (16 per cent and 207 per cent for the extreme cases). Examples of even greater variation of recurrence interval (40 to 300 years) for large ruptures with similar amount of slip exist for segments of the San Andreas fault[5]. Perhaps the whole simple model of recurrence of great earthquakes may have to be substantially revised because the energy release in re-ruptures and the recurrence time vary greatly.

The main value of Thatcher's work is that he has pointed out the inadequacy of a simple model of recurrence that was easily understood and seemed to make sense. The more complicated model he has proposed[1,2] for explaining the varied nature of re-rupture may not convince all readers. He has not considered the possibility of asperities (strong spots at the fault surface) changing their role from barriers to rupture initiation points as a function of the strain accumulated in them. Also, the amount of slip variation in one earthquake along the fault is not discussed quantitatively, leaving the reader wondering whether a 30 per cent difference in slip is enough to separate asperities from weak fault segments, and how much aseismic fault creep is necessary in Thatcher's model to balance the long-term plate motion along the boundary.

It is not particularly comfortable when 'well established', simple and sensible models are discredited. Thatcher has done this and raised additional questions, which may lead to more changes of the recurrence model of great earthquakes. As Thatcher emphasizes, the role of asperities[6-10], their distribution and their activation in subsequent cycles must become clear before we can fully understand the rupture process along great faults and make substantial progress in predicting earthquakes. ☐

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1. Thatcher, W. *Nature* **341**, 432–434 (1989).
2. Thatcher, W. *J. geophys. Res.* **95**, 2609–2623 (1990).
3. Bakun, W. H. & McEvilly, T. V. *J. geophys. Res.* **89**, 3051–3058 (1984).
4. Wyss, M. *Science* **234**, 726–728 (1986).
5. Sieh, K., Stuiver, M. & Brillinger, D. *J. geophys. Res.* **94**, 603–623 (1989).
6. Wesson, R. L., Burford, R. O. & Ellsworth, W. L. *Proc. Conf. Tectonic Problems of the San Andreas Fault System* (eds Kovach, R. L. & Nur, A.) 303–321 (Stanford University, Stanford, 1973).
7. Aki, K. *J. geophys. Res.* **84**, 6140–6148 (1979).
8. Lay, T. & Kanamori, H. in *Earthquake Prediction* (eds Simpson, D. W. & Richards, P. G.) 579–592 (Am. Geophys. Union, Washington, D.C., 1981).
9. Wyss, M., Johnston, A. C. & Klein, F. W. *Nature* **289**, 231–234 (1981).
10. Aki, K. *J. geophys. Res.* **89**, 5867–5872 (1984).

PRIMATOLOGY

Some relatives take a dive

R. D. Martin

BECAUSE of their very obscurity, the earliest origins of modern primates remain a topic rooted more in conjecture than in fact. The reports by Beard[1] and by Kay *et al.*[2] on pages 340 and 342 of this issue, concluding that one family of so-called 'archaic primates' can be ruled out of line, provide much-needed evidence and at the same time give cause for a fresh round of speculation.

By mid-Eocene times, 45 million years ago, prosimian primates of modern aspect were well established, being documented by associated skulls and skeletons from both North America (for example *Notharctus*[3]) and Europe (*Europolemur*[4] and *Pronycticebus*[5]). By this stage, many characters typical of modern primates — large, forward-facing eyes surrounded by a complete bony rim, hindlimb domination and grasping feet — had developed and a great diversity of species was already present. Indeed, evidence from the Fayum site (Egypt) indicates that even advanced simian primates had evolved by the end of the Eocene[6]. Hence, the common ancestor of modern primates undoubtedly dates back well into the Palaeocene, some 60 million years ago, if not earlier; but Palaeocene primates of modern aspect have yet to be documented. Instead, Palaeocene deposits of North America and Europe have yielded four families of so-called 'archaic primates' (Plesiadapidae, Paromomyidae, Picrodontidae, Saxonellidae), which are usually combined in the infraorder Plesiadapiformes of the order Primates[7–9]. These fossils resemble later primates in certain features of the molar teeth and the ear region, but lack many other typical primate characters. Some authors have therefore questioned the inferred phylogenetic link between plesiadapiforms and primates[10–12].

So far, this issue has remained contentious because only the single genus *Plesiadapis* was known from reasonably well-preserved skulls and skeletons[8,9,13]. The picture has now radically changed following the discovery of new material from two late-surviving genera of the family Paromomyidae in earliest Eocene deposits of Wyoming. Here Beard[1] reports on two partial skeletons of *Phenacolemur*, and Kay and colleagues[2] describe a virtually complete skull of *Ignacius*. Both Beard and Kay *et al.* reach the same revolutionary conclusion — that paromomyids are related not to primates but to the Dermoptera, a peculiar order of Southeast Asian gliding mammals containing only two species (genus *Cynocephalus*).

Lithograph of a living 'flying lemur' (Dermoptera) from *Histoire Naturelle des Singes et des Makis*, 1800. The plate shows the animal's elongated middle digits, also found by Beard in his fossil specimens, which are necessitated by the extension of the gliding membrane on to the hands and feet. (Although dermopterans are commonly referred to as flying lemurs, as George Gaylord Simpson pointed out they are not lemurs and they do not fly!)

The partial skeletons discussed by Beard were associated with dental remains, permitting them to be identified with confidence as *Phenacolemur*. As a spin-off, isolated postcranial elements could be allocated to *Ignacius* and *Phenacolemur*. In both, the middle phalanges (digital bones) of the fingers and toes are markedly longer than the basal phalanges. This unusual condition is lacking among primates, living or fossil. Among modern mammals, dermopterans provide the only counterpart: the gliding membrane (patagium) extends onto the digits of the hands and feet, which explains the elongated middle phalanges. Beard also identified derived features of the wrist and ankle, which suggest a link between *Phenacolemur*, *Ignacius* and *Cynocephalus*, and he concludes that Paromomyidae and Dermoptera are sister groups.

Kay et al. show that the skull of *Ignacius* shares certain derived features with dermopterans and differs distinctly from primates. One diagnostic feature of primates of modern aspect is formation of the auditory bulla primarily as an outgrowth of the petrosal bone[8,9,12]. In *Ignacius*, by contrast, the bulla is apparently formed by a separate element, an entotympanic. Re-examination of the evidence for *Plesiadapis* indicates that in this genus, too, the bulla is formed by an entotympanic. The inferred presence of a petrosal bulla in *Plesiadapis* has provided one of the main arguments for a phylogenetic link between plesiadapiforms and primates. But it is notoriously difficult to determine the composition of the bulla in adult mammals because of fusion of sutures[14]. (Tree-shrews, once thought to have a petrosal bulla, provide a classic example — from developmental evidence, it eventually became clear that the bulla is formed from an entotympanic that later fuses with the petrosal.)

Kay *et al.* also infer that *Plesiadapis*, *Ignacius* and *Cynocephalus* share an unusual derived feature in that the internal carotid system is only poorly developed, the principal intracranial blood supply being provided through the vertebral arteries. They therefore go further than Beard and propose that the entire infraorder Plesiadapiformes represents the sister-group of the Dermoptera.

The new evidence opens up several possibilities. The first is that Dermoptera and Primates are related and that the Plesiadapiformes represent an important connecting link between them. This would fit the idea, now experiencing a renaissance, that tree-shrews, bats, dermopterans and primates constitute a phylogenetic unit (the 'Archonta'). On the other hand, resemblances between these four groups may simply reflect retention of primitive features from ancestral placental mammals[14]. A second possibility (left open by Beard, but ruled out by Kay *et al.*) is that plesiadapids are related to primates of modern aspect, while paromomyids are related to dermopterans. But the evidence for a link between plesiadapids and primates is considerably weakened if the former have an entotympanic bulla.

Third, as Kay *et al.* suggest, the entire infraorder Plesiadapiformes may be specifically related to dermopterans. Evidence from the intracranial blood supply supports this interpretation, but there is a problem with the bulla — Kay *et al.* cite fusion of a collar-shaped ectotympanic to the outside of the bulla as a shared derived feature of plesiadapiforms and dermopterans, yet Gingerich[9] has described a well-preserved skull of *Plesiadapis* that clearly shows an ectotympanic ring enclosed within the bulla. Finally, it could be that plesiadapiforms are connected neither with primates nor with dermopterans, and that the paromomyids convergently developed gliding adaptations paralleling those of modern dermopterans.

As dental evidence has figured so prominently in discussions of the purported link between plesiadapiforms and primates, it is curious that no specific dental resemblances between *Cynocephalus* and plesiadapiforms have been proposed. Whatever the outcome, the new evidence challenges the long-accepted relationship between plesiadapiforms and primates, and it shows that paromomyids, at least, were sky-divers. ☐

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1. Beard, C. *Nature* **345**, 340–341 (1990).
2. Kay, R., Thorington, R. & Houde, P. *Nature* **345**, 342–344 (1990).
3. Gregory, W. K. *Mem. Am. Mus. nat. Hist.* **35**, 239–257 (1920).
4. Franzen, J. L. *Cour. Forsch.-Inst. Senckenberg* **91**, 151–187 (1987).
5. Thalmann, U., Haubold, H. & Martin, R. D. *Palaeovertebrata* **19**, 115–130 (1989).
6. Simons, E. L. *Science* **247**, 1567–1569 (1990).
7. Simons, E. L. *Primate Evolution: An Introduction to Man's Place in Nature* (Macmillan, New York, 1972).
8. Szalay, F. S. & Delson, E. *Evolutionary History of the Primates* (Academic, New York, 1979).
9. Gingerich, P. D. *Mus. Pal. Univ. Mich., Pap. Paleont.* **15**, 1–140 (1976).
10. Cartmill, M. in *The Functional and Evolutionary Biology of Primates* (ed. Tuttle, R.) 45–77 (Aldine-Atherton, Chicago, 1972).
11. Martin, R. D. in *Major Topics in Primate and Human Evolution* (eds Wood, B. A., Martin, L. B. & Andrews, P. J.) 1–31 (Cambridge University Press, 1986).
12. Martin, R. D. in *The Study of Prosimian Behaviour* (eds Doyle, G. A. & Martin, R. D.) 45–77 (Academic, New York, 1979).
13. Szalay, F. S., Tattersall, I. & Decker, R. L. *Contrib. Primatol.* **5**, 136–166 (1975).
14. Martin, R. D. *Primate Origins and Evolution: A Phylogenetic Reconstruction* (Chapman & Hall, London/Princeton University Press, 1990).

OXIDE SUPERCONDUCTORS

Contrasting critical currents

Neil Alford

APPLICATIONS of high-temperature superconductors have so far been severely limited because their current densities are disappointingly low. To be useful, these materials need to have zero resistance at liquid-nitrogen temperatures or higher, with large current densities in high magnetic fields, and the samples need to be large (of the order of metres) for use as wires or cables, for example. On page 326, Ru Ling Meng *et al.*[1] describe a 'continuous' process for making $YBa_2Cu_3O_x$ with high current densities. The process is continuous in the sense that the sample is moved through a hot zone in a furnace and directional solidification is encouraged. In principle, such a process may enable samples of large dimensions with controlled grain orientation to be made, although, as yet, the samples are only centimetres in length.

There are three important factors that limit the current-carrying capacity of the bulk high-temperature superconductors: the presence of weak links at grain boundaries; the anisotropy in the current-carrying ability in crystals of the material — best in the *a–b* plane and poor in the *c*-axis direction; and the weakness of flux pinning. In thin films, experiments at IBM[2] have shown that although the critical-current density, J_c, within a single grain can be of the order of millions of amps per cm^2, the inclusion of a grain boundary and misorientation between crystals can cause a drastic reduction in J_c of about two orders of magnitude.

The first to achieve high critical-current density, J_c, in bulk $YBa_2Cu_3O_x$ (123) was Sungho Jin at Bell Labs[3], who showed that, by growing large single crystals, J_c could be increased from the usual 1,000 A cm^{-2} to over 17,000 A cm^{-2} (at temperatures of 77 K in zero magnetic field). More importantly, the J_c of such materials was less affected by magnetic fields than conventionally sintered, unoriented material. Murakami *et al.*[4] then showed that by melting, quenching and then annealing the $YBa_2Cu_3O_x$, exceptionally high J_c could be achieved even in very high magnetic fields of up to several tesla. Salama *et al.*[5] at Houston have confirmed these results and obtained a current density of 37,000 A cm^{-2} in a magnetic field of 0.6 T by direct (pulsed) measurement. A number of other groups (see, for example, refs 6, 7 and 8) have now obtained high values of J_c in small samples. All these methods make use of the liquid phase in the peritectic regime. Indeed, Murakami *et al.* believe that the problem of weak flux pinning may be overcome by the presence of the second-phase material which forms as a consequence of these peritectic growth methods. The peritectic region is one where a solid of composition A and a liquid of composition B combine to form a solid on cooling of composition C. The 123 compound will grow from a liquid in this region and may form as a single-phase material, but from experience second-phase particles such as Y_2BaCuO_x (211) form. If the melt is quenched, very fine particles of Y_2O_3, for example, may also remain. So why is the second-phase impurity useful?

A perfect crystal of high-T_c superconducting material has zero J_c, the exact opposite of what is required. Only when defects, which will pin the magnetic flux, are introduced will current be carried without the flux lattice disappearing. This is because the Lorentz force between the transport current and the flux lines causes the latter to move, thus generating a voltage, unless the flux lines are pinned. The defects are really the glue which holds the lattice together and without them there can be no transport of current. These defects must be of the order of the coherence length, which in the new oxide superconductors is extremely small — about 3 nm. The process of using a liquid phase in the peritectic region naturally produces second-phase material, and it is possible that by quenching, the dimensions of these second-phase particles can be constrained to the dimensions of the coherence length. Defects can also be introduced by irradiation and groups at Argonne, AT & T Bell Laboratories and Houston have obtained large increases in J_c in this way.

In the 'powder in a single tube' approach, the superconductor of choice is Pb-doped Bi–Sr–Ca–Cu–O (usually the three-layer compound 2223) because of the 'platey', mica-like particle morphology[9]. The swageing (reducing the cross-section by force) and rolling process used is standard metallurgy and the results, J_c = 32,000 A cm^{-2} at 77 K in zero field, 23,000 A cm^{-2} in 0.1 T and 6,000 A cm^{-2} in 1 T at 77 K are impressive. A uniform response can be maintained over several centimetres, and even at lengths of 2–3 m a J_c of 10,000 A cm^{-2} at 77 K in zero field can be attained (Ken Sato of Sumitomo Electric, personal communication). The microstructure of these materials shows excellent alignment of the *a–b* plane and one can imagine that the repeated sintering and rolling steps gives good intergranular contact thereby reducing the weak-link problem. Interesting work by Vacuumschmelze in Germany shows that silver-clad $Bi_2Sr_2CaCu_2O_x$ has high J_c which is not affected by the presence of magnetic fields, with properties at 4 K rivalling those of conventional superconductors. Unfortunately, however, at 77 K the properties are poor.

New work at Southampton University is also throwing up some exciting indications of what may be possible[10]. This group finds that the transport J_c of melt-processed $YBa_2Cu_3O_x$ is 16,000 A cm^{-2} at 0 T and 77 K and 2,000 A cm^{-2} at 1 T and 77 K. What is interesting is that transport a.c. measurements are much higher, J_c = 12,000 A cm^{-2} at 5 T and 77 K measured at 50 Hz.

The possibility of continuous processing raised by Meng *et al.*[1] brings practical applications for high-T_c superconductors closer to realization, because of the potential to maintain high J over useful lengths

It also seems likely that such material will have low surface resistance and so it could be useful in the area of radio-frequency and microwave applications.

Meng *et al.* measured a transport J_c at 77 K in magnetic fields and obtained 7,500 A cm^{-2} at 0.82 T parallel to the direction of transport and 14,000 A cm^{-2} perpendicular to it. The material shows, therefore, far better field dependence than conventionally sintered materials where, at 0.82 T, the J_c is less than 10 A cm^{-2}. The materials are comparable, or possibly slightly inferior, to the materials made by Murakami or Salama using a melt and quench process — this, however, is a batch, not a continuous process.

What are the prospects? All the work on melt-processed samples mentioned here has been undertaken on what can only be described as tiny samples, and cables kilometres in length with high J_c are still a long way off. Moreover, there are other problems yet to solve. For example, the kinetics of the crystallization process dictate that it is bound to take a long time and then because the material contains no interpenetrating porosity the oxygenation required could be prohibitively long[11]. The fracture toughness will still be that of glass so handling may be a problem. But science is generally hard graft interspersed with the occasional breakthrough. We have seen that in the high-temperature superconductors the properties are there for the taking. The paper from Professor Chu's group in this issue is an indication of what may be possible. ☐

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1. Meng, R.L., Kinaldis, C., Sun, Y.Y., Gao, L., Tao, Y.K., Hor, P.H. & Chu, C.W. *Nature* **345**, 326–328 (1990).
2. Chaudhari, P. *et al. Phys. Rev. Lett.* **60**, 1653 (1988).
3. Jin, S. *et al. Phys. Rev.* B**37**, 7850 (1988).
4. Murakami, M. *et al. Jap. J. appl. Phys.* **28**, 1189–1194 (1989).
5. Salama, K. *et al. Appl. Phys. Lett.* **54**, 2352–2354 (1989).
6. McGinn, P.J. *et al. Physica* C**156**, 57–61 (1988).
7. Alford, N. *et al. J. appl. Phys.* **65**, 2856–2858 (1989).
8. Pollard, R.J. *et al. Supercond. Sci. Technol.* **2**, 169–172 (1989).
9. Hikata, T. *et al. Jap. J. appl. Phys.* **28**, 882–L84 (1989).
10. Yang, Y. *et al. Supercond. Sci. Technol.* (in the press).
11. Alford, N. *et al. Nature* **332**, 58–59 (1988).

ELECTROCHEMISTRY

More efficient solar cells

Nathan S. Lewis

As THE search continues for an inexpensive, efficient solar-energy-conversion device, conventional photovoltaic devices are under increasing competition from 'wet' photoelectrochemical cells. Photoelectrochemical cells are relatively simple (and potentially inexpensive) to construct, because they require merely the immersion of the desired photoactive solid into an ionically conducting liquid. On page 330, a team led by S. Licht[1] describe how in certain cases it is possible to achieve the desirable combination of high efficiency and low corrosion rates by manipulating the composition of the conducting liquid. With their approach, Licht *et al.* demonstrate that cadmium selenide electrodes can be coaxed into stable operation in an aqueous solution with an overall energy-conversion efficiency of 17 per cent.

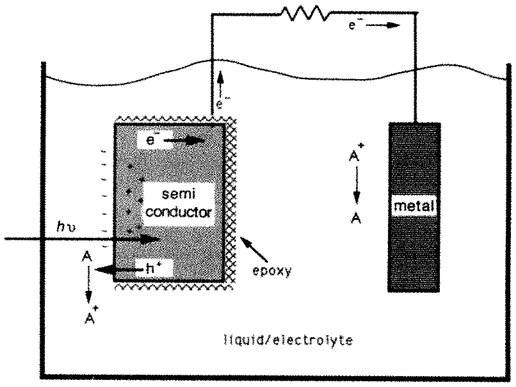
In the general scheme of energy-conversion technologies, semiconductor/liquid cells fall in between solid-state photovoltaics and artificial photosynthetic systems. The solid-state systems are the most advanced, efficient and reliable devices, with some specialized laboratory devices yielding terrestrial efficiencies in the 25–26 per cent range. But their efficiency comes at a price, both in terms of the expense in making the material and the complex processing steps used to coax out the last percentage points in efficiency. At present, semiconductor/liquid systems offer efficiencies in the 10–15 per cent range, with demonstrated lifetimes in several systems now ranging from months to years. The molecular-based artificial photosynthetic systems are the least efficient and stable, with efficiencies on the order of one per cent and stability limited by molecular photodegradation processes. But the molecular systems do at least offer an opportunity for chemical design of the entire system, which might eventually enable construction of a photoresponsive assembly that is analogous to the tried-and-true method of natural photosynthesis.

The principles leading to photocurrent flow at a semiconductor/liquid junction, like that used by Licht *et al.*, are very similar to those of conventional photovoltaic cells (see figure). The junction, between the solid and liquid produces an electric field, which separates photogenerated charge carriers, electrons and holes. Carrier recombination can be minimized, and efficient chemical energy conversion is possible, if these charge carriers can be transferred across the solid/liquid interface and onto the desired ions in the liquid. Manipulation of the reactions that occur at the two electrodes in the photoelectrochemical cell can result in the production of electricity, fuels or both[2].

One of the key problems plaguing the development of these systems is the trade-off between the stability of the solid and the efficiency of the resulting device: semiconductors that are stable in the presence of light often make inefficient photovoltaic devices, whereas the alternative solid/liquid combinations generally corrode when illuminated. Several strategies are being used to tackle this problem — non-aqueous solvents can be used to suppress corrosion; novel semiconductors are being developed that are more photochemically inert in aqueous solutions; the surface of traditional semiconductors is being modified chemically; and manipulation of the composition of the ions in the aqueous electrolyte is being investigated. All these methods have particular advantages, but it is not yet clear which strategy is most effective.

In Licht's work, the authors have used cadmium selenide as the photoanode in an aqueous cell containing hexacyanide complexes of Fe(II) and Fe(III) as the charge carriers. They find that at specific values of the Fe(II) and the Fe(III) concentrations, the photoanode is stable under illumination for greater than 5,000 Coulombs cm^{-2} of total charge flow. Specific concentrations are required to achieve a fine balance between the diffusive flux of ions to the surface, the need rapidly to capture the photogenerated charges before they have a chance to participate in corrosion processes, and the tendency to deposit insulating layers of chemical films if the solubility of the reaction products is exceeded. Achieving this delicate balance in the aqueous cadmium selenide system has allowed Licht *et al.* to improve the cell stability from seconds to days, while simultaneously achieving excellent output properties.

The magnitude of the voltage developed in these cells is possibly even more impressive than their improved stability. Many workers associate the performance of semiconductor/liquid junctions with that of semiconductor/metal (Schottky) contacts. Unfortunately, Schottky contacts are notoriously inefficient photovoltaic devices, because the metal overlayer efficiently collects both electrons and holes. This leads



Schematic of a photoelectrochemical cell. Light absorbed by the semiconductor electrode is converted into a flow of electrical charge in the external circuit.

to excessive recombination as compared to p–n junction-type photovoltaic devices. Early work indicated that excessive recombination was also prevalent at solid/liquid interfaces, but more recent studies have shown that junction recombination can be greatly suppressed with the proper choice of semiconductors and redox ions. In fact studies of silicon/liquid interfaces have shown that extremely high open-circuit voltages can be obtained, even by comparison to well-developed silicon solid-state photovoltaic cells[3]. A similar situation appears to be occurring in the cells of Licht *et al.*. Their cells develop photopotentials of 1.2 V, and the bandgap energy is 1.7 electron volts (eV). This ratio is very close to the upper limit that could be achieved from a material with a 1.7-eV band gap, again indicating that near-optimal performance can be achieved in certain semiconductor/liquid combinations. The ability to reach this voltage is even more impressive given the constraints on corrosion suppression, film formation and interfacial kinetics that must be addressed simultaneously in the cadmium selenide system.

One of the unknown features of this sytem is the rate at which charge carriers actually cross the interface. An understanding of these rates is important, because rapid capture of carriers will allow effective competition with both recombination and corrosion. The few kinetic measurements that have been performed to date on semiconductor/liquid junctions have yielded a wide disparity in the timescales for the charge-transfer process, with values ranging from picoseconds to milliseconds. The factors that control these rates are not well understood at present, and extensive research is still required in order to obtain interfaces with the desired kinetic properties.

In contrast to extraction of energy from fossil fuels or nuclear power, active uses of solar energy are unique in that they do not directly contribute to global warming. As global warming issues gain the attention of the public and policymakers, and more attention is paid to evaluating the true cost of fossil fuels, solar energy processes should regain lost attention when considering alternative energy options. Whether the specific solar technology involves semiconductor/liquid junctions, solid-state systems, artificial photosynthetic devices or some hybrid thereof remains to be seen. ☐

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1. Licht, S. *et al. Nature* **345**, 330–333 (1990).
2. *Photocatalysis: Fundamentals and Applications*, (eds Serpone, N. & Pelizzetti, E.) (Wiley, New York, 1989).
3. *Semiconductor Electrodes* (ed. Finkles, H.O.) (Elsevier, Amsterdam, 1988).

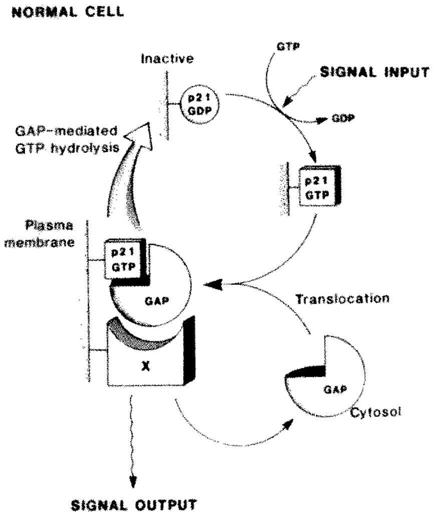
CRYSTALLOGRAPHY

Following proteins in time

Louise N. Johnson

UNLIKE conventional X-ray crystallography, Laue protein crystallography, which uses the bright, broad spectrum of X-radiation emitted by synchrotron radiation sources, has great potential for time-resolved studies on the dynamic properties of proteins in the crystalline state. In 1984 it was demonstrated that the method (white X-ray beam: stationary crystal) was feasible for proteins[1,2], and in 1987 that it could be applied to elucidate ligand binding[3]. Now, on page 309 of this issue, Schlichting *et al.*[4] show that the method can provide time-resolved information about a biologically important molecule. The authors report a detailed structural study using the DESY synchrotron, Hamburg, on the active Ras p21·GTP complex and a complex on the pathway to the product p21·GDP complex. The hydrolysis of GTP proceeds with a slow half-life (about 40 min in the crystal, similar to that in solution) and the first image of the enzyme·GTP complex was obtained 4 min after starting the reaction with a total exposure of 45 s. The p21 crystals are sensitive to radiation damage; Schlichting *et al.* obtained only two data sets for each crystal, and only one in ten crystals was sufficiently well ordered. The method is not without its technical difficulties.

The advances in Laue crystallography[5] have depended on advances in physics and in chemistry. The physics has been necessary not only in the design and operation of synchrotron radiation sources and protein crystallography stations, but also for the development of optimum protocols and software to obtain reliable intensity estimates from the diffraction photographs. Advances in chemistry, in particular the development of caged compounds[6,7], have allowed the start of the reaction to be synchronized with the start of the collection of X-ray data. Here, the substrate is made biologically inert through covalent attachment of a photolabile protecting group (Schlichting *et al.* used a 2-nitrophenyl ethyl group). Illumination by a xenon flash lamp results in dissociation of the protecting group and liberation of the substrate. Such strategies have been employed previously in the study of muscle ATP hydrolysis and neurotransmitter mechanisms.



A scheme for p21 activity and function (from ref. 10, with permission).

Schlichting *et al.* co-crystallized the p21 protein in the presence of caged GTP and elucidated the structure by conventional date collection methods. After illumination (ten flashes from a xenon flash lamp over about 1 min), the authors studied the resulting active GTP complex some 4 min and 14 min after reaction by Laue crystallography and after 19 h by conventional methods. Even though only 50 per cent of the data to 2.8-Å resolution were obtained with the Laue method, the electron-density maps and the crystallographic refinement of the structures were sufficiently well-defined to give precise information about the complexes. In agreement with analysis of the complexes using high-performance liquid chromatography[8], the image after 4 min was compatible with a GTP complex, that after 14 min with a mixed state in which there had been hydrolysis, and that after 19 h was consistent with the GDP complex. Interestingly, the caged GTP molecule, bound in a nonproductive mode, was able to rearrange in the crystal after liberation of the cage to yield the active GTP complex.

What does this teach us about the biological function of the Ras p21 protein? The family of *ras* genes in the mammalian genome has been intensely studied following the identification of their transformed alleles in about 10 per cent of human cancer tumours[9]. The *ras* genes acquire their transforming properties by single point mutations in their coding sequences corresponding to positions 12, 13, 59 and 61 of the expressed protein p21. The cellular role of the protein is not known, but by analogy to the G proteins in signal transduction pathways and the bacterial elongation factor Tu, p21 is thought to function by exchanging GTP for GDP in response to an extracellular signal. The p21·GTP complex is then recognized by the GTPase-activating protein (GAP) and the resulting complex acts as a signal for growth[10] (see figure). In the normal cell, the signal is switched off by p21-catalysed hydrolysis of GTP, the catalytic activity being greatl increased by interaction with GAP. In the mutant proteins, GTPase activity is low and is not increased by GAP. So the protein remains in the 'on' state, prolonging a signal for unregulated cell growth.

Precise information about the structural changes has been provided by the Berkeley group for GDP and inactive GTP analogue complexes[11], by the Heidelberg group[12] for the refined structure of an inactive GTP analogue complex (now at 1.35-Å resolution[13]), and by the new work[4] for active GTP and GDP complexes. The recombinant p21 proteins differ slightly – residues 1–166 (Heidelberg); residues 1–177 (Berkeley) – and both lack the C-terminal residues involved in attachment of the protein to the cell membrane. The proteins crystallize in different space groups and the crystallographic analysis therefore provides several independent views of the molecule.

Although a detailed comparison of the proteins has still to be made, some common features are apparent. Both groups describe a similar constellation of the amino acids that contribute to the binding of the base, ribose and α-phosphate components of the guanine nucleotides and there is negligible change in these between the GTP and GDP complexes. In the GTP complexes, the β- and γ-phosphates are doubly coordinated by the magnesium ion and by the NH_3^+ group of Lys 16. In addition, the main-chain NH of Thr 35 and Gly 60 hydrogen-bond to the γ-phosphate.

There are differences in detail that may be important. Schlichting *et al.*[4] report an interaction between Gln 61 and the γ-phosphate through a water molecule, and in the active GTP complex there is an additional hydrogen bond between the β–γ bridging oxygen and the main-chain NH of Gly 13. (GTP has 10–100 times the affinity of the inactive analogue for p21). The main changes in structure between the p21·GTP complexes and the p21·GDP complex occur in two loop regions, L2 (movements of residues 32–38) and L4 (movements of residues 60–65). Both regions are involved in the recognition of the γ-phosphate. Residues 32–36 have been implicated in GAP recognition by mutational experiments[14]. The catalytic mechanism, which involves attack by a water molecule[15], is not yet known, but is likely to involve Lys 16 in transition-state stabilization, Gln 61 in the attack by water, and Thr 35 and Asp 57 in the magnesium and water coordination[13]. At present there is no outstanding candidate for promoting activation of the water, unlike (for example) phosphofructokinase, where the role of Asp 127 is clear[16].

Why are the mutant proteins not activated by GAP and why do they have negligible GTPase activity? Surprisingly, comparison of the p21 mutant (Gly 12 replaced with Val) and native GTP complexes shows similar binding modes of GTP and only small differences near the loop 10 to 18 that wraps around the phosphate-recognition site. There are differences, however, in conformation of residues Gly 60, Thr 38 and Gln 61 that are involved in the γ-phosphate recognition site, and these changes are likely to affect catalysis. ☐

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1. Moffat, K., Szebenyi, M.E. & Bildercack, D.H. *Science* **223**, 1423–1425 (1984).
2. Helliwell, J.R. *Rep. Prog. Phys.* **47**, 1403–1497 (1984).
3. Hadju, J. *et al. Nature* **329**, 115–116 (1987).
4. Schlichting, I. *et al. Nature* **345**, 309–315 (1990).
5. Hajdu, J. & Johnson, L.N. *Biochemistry* **29**, 1669–1678 (1990).
6. Kaplan, J.H., Forbush, B. & Hoffman, J.F. *Biochemistry* **17**, 1929–1935 (1978).
7. McCray, J.A. & Trentham, D.R. *A. Rev. Biophys. Chem.* **18**, 239–270 (1989).
8. Schlichting, I. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 7687–7690 (1989).
9. Barbacid, M. *A. Rev. Biochem.* **56**, 779–827 (1987).
10. McCormick, F. *Cell* **56**, 5–8 (1989).
11. Milburn, M.V. *et al. Science* **247**, 939–945 (1990).
12. Pai, E.M. *et al. Nature* **341**, 209–214 (1989).
13. Pai, E.M. *et al. EMBO J.* (in the press).
14. Gibbs, J. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 5026–5030 (1988).
15. Feuerstein, J., Goody, R.S. & Webb, M.R. *J. biol. Chem.* **264**, 6188–6190 (1989).
16. Shirakihara, Y. & Evans, P.R. *J. molec. Biol.* **204**, 973–994 (1988).

BIOGEOCHEMICAL CYCLES

El Niño and atmospheric CO_2

Ulrich Siegenthaler

ON average, the amount of carbon dioxide in the atmosphere is steadily increasing by about 1.5 parts per million (p.p.m.) per year. The increments from one year to the next are, however, not constant but fluctuate around a mean trend. These fluctuations are associated with El Niño/Southern Oscillation (ENSO) events. During an ENSO event, the sea surface temperatures in the tropical Pacific Ocean increase and the upwelling of nutrient-rich water ceases, thereby increasing the uptake of carbon dioxide from the atmosphere by the ocean. The apparent link between ENSO events and fluctuations in atmospheric carbon dioxide concentrations seems plausible. The simplicity of this argument is, however, misleading. C. D. Keeling and others[1] have carefully analysed measurements of the concentration and $^{13}C/^{12}C$ ratio ($\delta^{13}C$ value) of atmospheric carbon dioxide and find that biospheric, as opposed to oceanic, processes are the primary cause of the interannual fluctuations in atmospheric carbon dioxide.

To study the interannual fluctuations in CO_2, Keeling *et al.* first filtered out the seasonal cycle of CO_2 from the records, then they removed the long-term increasing trend by subtracting a constant fraction of the cumulative fossil CO_2 emissions. Figure 1 shows the average of the resulting anomalies in CO_2 concentrations over Mauna Loa and the South Pole; this curve shows, to a first approximation, fluctuations of the global mean atmospheric CO_2 content. The correlation of the CO_2 anomalies with ENSO events is obvious: during such an event, a rise in CO_2, exceeding the normal anthropogenic growth rate, starts and lasts for about a year. Before such an increase, a dip is sometimes observed. The interannual CO_2 variations must reflect imbalances in the exchange fluxes between the atmosphere and the ocean and/or the terrestrial biosphere. Although the oceanic and biospheric sources of CO_2 cannot of course be distinguished by examining the concentration data alone, they can be distinguished by looking at the $\delta^{13}C$ value of the atmospheric CO_2. Carbon dioxide released from the ocean has nearly the same $\delta^{13}C$ as atmospheric CO_2 (about −8‰), because atmospheric CO_2 is in approximate isotopic equilibrium with dissolved inorganic carbon in sea water. In contrast, CO_2 of biospheric origin typically has a $\delta^{13}C$ of

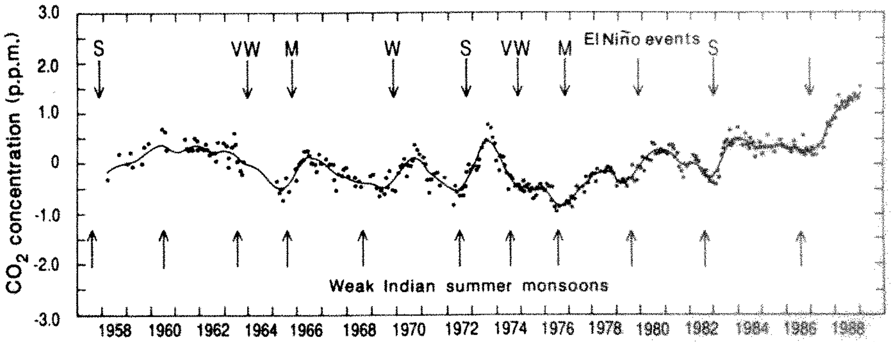

Fig. 1 Atmospheric CO_2 concentration anomaly obtained by Keeling *et al.*[1] by removing the seasonal variations as well as the secular trend from their observational records. The data points represent averages of the anomalies of Mauna Loa and the South Pole. The strength of the El Niño events is indicated, ranging from very weak (VW) to strong (S).

$-25‰$ and is thus clearly distinguishable from that originating from the ocean.

Figure 2 shows a seasonally adjusted record of $\delta^{13}C$. Excursions towards lower values of $\delta^{13}C$ (notice inverted scale) are observed in 1983–84 and 1987–88 and also 1979–80, occurring at the same time as the anomalous increases in CO_2 (Fig. 1) after an ENSO event. The anomalies are small, comparable to the analytical precision, but it seems unlikely that they are an experimental artefact, as they are found in the individual isotopic records for both Mauna Loa and the South Pole. Thus, the isotope data suggest that the positive CO_2 anomalies are of biospheric origin.

Examining both the isotopic records and the records of CO_2 concentration allows both the biospheric and oceanic sources to be identified: from the anomalies in CO_2 concentration, the sum of biospheric and oceanic sources (or sinks) can be estimated, and anomalies in $\delta^{13}C$ allow the size of the biospheric contribution alone to be established. Keeling *et al.* have carried out a double deconvolution of the two records and obtained estimates for the 'anomalous' oceanic and biospheric CO_2 fluxes. Both fluxes are found to be large, up to 4 Gt C per year, and are out of phase: namely, biospheric release and oceanic uptake of carbon occur during and after an ENSO event, with the biospheric flux dominating, thus causing a net release of CO_2 to the atmosphere.

Is this result realistic? Qualitatively, it is certainly plausible. Ordinarily, surface waters in the equatorial Pacific Ocean have a high partial pressure of CO_2 (P_{CO_2}), with values 50–100 p.p.m. higher than in the atmosphere. This peak in P_{CO_2} is due to the upwelling of CO_2-rich water from depths of 100 m to a few 100 m and the resulting degassing from the equatorial Pacific corresponds to a source of about 1 Gt C per year. During an El Niño event, upwelling stops and therefore the CO_2 outgassing ceases. Although this is directly supported only by a few P_{CO_2} measurements during the 1982 event, there is clear evidence from data on total carbon and on nutrients that P_{CO_2} must have decreased. This is confirmed by box model studies[2,3] in which the physical, chemical and biological changes in the Pacific Ocean during an ENSO event are simulated. During such an event, the upwelling of water rich in CO_2 and nutrients into the equatorial surface mixed layer ceases; the temperature of the surface water increases and biological activity decreases (because of reduced availability of nutrients), as does the export of carbon by sinking organic particles. These last two effects would tend to increase P_{CO_2} in the surface water, but the first — which dominates — leads to a decrease of P_{CO_2}. Although the two model studies[2,3] differ in the details, both obtain the same decrease, about 0.4 p.p.m. (or 0.8 Gt C), in atmospheric CO_2 during an El Niño. The pattern of oceanic fluxes as predicted by the box models is in qualitative agreement with the pattern deduced by Keeling *et al.*, but their estimate of the oceanic CO_2 flux, up to 2 p.p.m. change within a year, is larger than can be explained by the models.

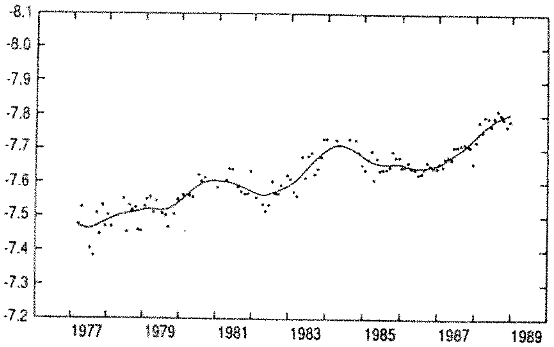

Fig. 2 Seasonally adjusted $\delta^{13}C$ values in atmospheric CO_2, average of results from Mauna Loa and the South Pole[1]. The vertical scale is inverted to facilitate comparison with Fig. 1.

The biospheric contribution also seems plausible. During an El Niño, rainfall is reduced in many tropical regions; and the correlation between El Niño and the weak southeast Asian summer monsoon is striking (Fig. 1). Consequently, there is apparently reduced uptake of CO_2 by vegetation over large areas, so that the anomalous atmospheric CO_2 increase during an El Niño seems essentially to be due to a reduced biospheric CO_2 sink.

In view of the relatively small CO_2 signals involved, one may wonder whether the story is relevant to discussions on the carbon cycle — the ENSO-related anomalies can hardly lead to changes in atmospheric CO_2 that would influence the greenhouse effect. The observed anomalies of 1–2 Gt C per year do, however, correspond to several per cent of the total terrestrial net primary productivity. This is a significant perturbation, particularly because it has its origin in only a restricted part of the land biota, and is important enough to be studied in more detail. As usual, what are needed are further long-term high-precision records of $\delta^{13}C$ and CO_2 concentration from stations near tropical ecosystems, and direct P_{CO_2} observations in the equatorial Pacific Ocean during an El Niño. ☐

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1. Keeling, C. D. *et al.* in *Aspects of Climate Variability in the Pacific and the Western Americas* (ed. Peterson, D. H.) *Geophys. Monogr.* **55**, 165–236 (Am. Geophys. Union, Washington, D.C., 1989).
2. Volk, T. *Global biogeochem. Cycles* **3**, 267–279 (1989).
3. Siegenthaler, U. & Wenk, T. in *Extended Abstr. 3rd Int. CO_2 Conference* Environmental Pollution Monitoring and Research Programme No. 59, 189–194 (World Meteorological Organization, Geneva 1989).

DAEDALUS

Purifying earth

LAST week Daedalus proposed a novel deep-sea mining scheme, in which two boreholes were sunk in the sea floor to such a depth that the hot pressurized water would dissolve the rock between them. Thereafter, a convective flow would be established, with cold sea water sinking down one of the holes, and emerging from the other very hot, and saturated with minerals in solution. He now plans to try the same scheme on land.

DREADCO prospectors are studying hot-spring districts in Iceland and New Zealand, and the geothermal regions of southwest England, where hot rock is very near the surface. A borehole in such a region should soon reach a depth where water would boil but for the strong hydrostatic pressure above it. The hotter and more pressurized the water, the more ferocious it becomes as a solvent. Once the hole has reached a certain critical depth, it need only be kept full of water and it will dissolve its way deeper and deeper without further drilling. Two holes a suitable distance apart will ultimately join up by sideways diffusion of the water. A self-sustaining convective flow between them could then be easily established.

This simple arrangement would be a wonderful source of geothermal heat and deep-dissolved minerals. But Daedalus also sees it as a splendid new waste-disposal and sewage-treatment plant. Sewage, possibly loaded with suspended urban waste and industrial nasties like chlorinated residues, would simply be poured into the downflow pipe. In the heated and pressurized depths, all the solids would dissolve in the water and would begin to react with it and the surrounding rocks. Some of the metals in the waste, like sodium and aluminium, would probably be trapped by ion-exchange processes; others, like iron, copper, and manganese from spent batteries, would go to stable sulphide or carbonate minerals for reclamation. The organics, from paper and plastics to food waste and sewage solids, should hydrolytically oxidize, ultimately to carbon dioxide. All the waste of civilization would be disposed of simply and with total ecological rectitude!

The final outflow, with luck, would be a sterile solution of minerals in almost pure water. Once the minerals had precipitated out and the dissolved gases had fizzed away, it might almost be fit to drink! But even Daedalus is not quite so optimistic. He would prefer to arrange the pressure-gradient in the outflow so that the exit-water partly boils on its way up. The steam, after driving turbines for power, could be condensed to pure distilled water for domestic distribution. The residual mineral-loaded stream, concentrated by its partial evaporation, could be filtered to recover its metals. David Jones

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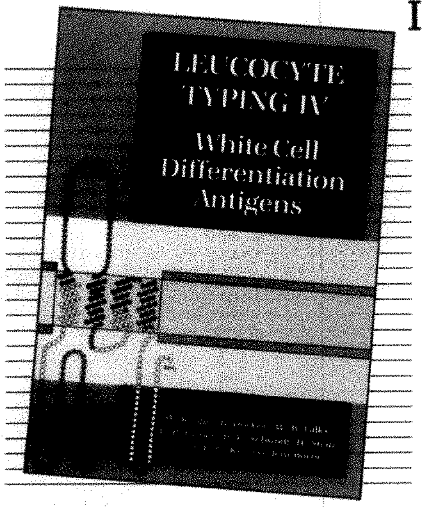
IMMUNOLOGY



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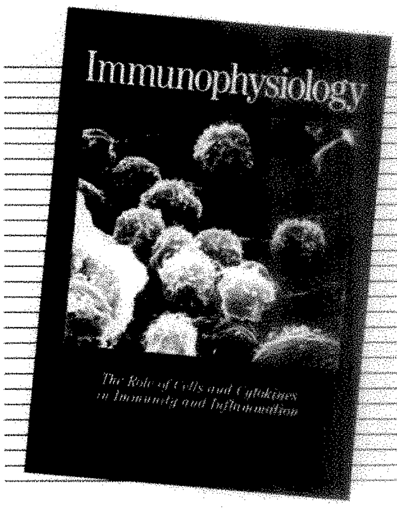
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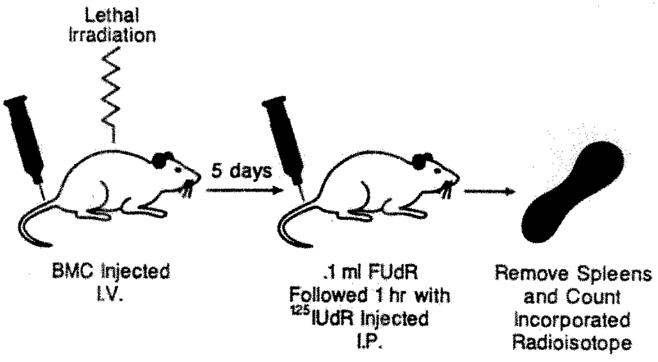

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Crystals from first principles

SIR—Maddox[1] considers it a "continuing scandal" that "it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition". In response, Cohen[2] has claimed that, on the contrary, "structures of crystals can be predicted using information about their chemical composition as the only input". Without wishing to underestimate the significance of the results discussed by Cohen, I feel that he has greatly overstated the case.

The stated aim of first-principles calculations is to predict the structure and properties of a crystal given only its chemical composition. If calculations are started from a configuration close to the global energy minimum, this may be done very successfully for a variety of materials[1,2]. Given the correct bond connectivity (or approximate relative atomic positions), cell size (in number of atoms) and (in some cases) space-group symmetry, the metric properties of the structure can be calculated, together with various properties of its energy and energy derivatives[1-4], but clearly these calculations require as input much more than simply the chemical formula. Although both Maddox and Cohen gloss over this problem, it is far from a trivial one.

There are two ways in which the calculations may be approached. First, an assemblage of randomly arranged atoms may be allowed to move such that the total energy of the system is minimized. This process can proceed with no assumptions (concerning, for example, which atoms are bonded to which, or what coordination numbers occur). The global energy minimum will be reached only if the derivative of the energy with respect to every variable parameter allows a continuous downward path towards it. From a random starting position, it is likely that the process will converge instead to a local minimum that represents a (possibly unphysical) metastable state. The calculations for silica[3] discussed by Maddox show that several polymorphs are dynamically stable and will 'trap' any minimization that starts within their energy volume of influence. The actual occurrence of such metastable forms indicates that there is not a continuously decreasing energy path between the metastable and the stable forms. Simulated annealing techniques[5] can permit escape from local minima, but are viable only if the number of local minima is small; this number is, however, usually very large. In general, therefore, solution is possible only by imposing strong boundary constraints, usually in terms of symmetry and cell size. For the former, a calculation starting with triclinic symmetry should converge on the correct symmetry. Imposing constraints on cell size is more difficult. Calculations can be performed for all possible cell sizes up to some arbitrary cutoff (with periodic boundary conditions), or for some large number of atoms (without periodic boundary conditions), where the expectation is that minimization will converge to an arrangement with the correct cell size and periodicity. In the latter case, the minimum number of atoms must be quite large because the properties of the assemblage must be those of a bulk crystal; experiments[6] suggest that this requires something of the order of 100–1,000 atoms.

The alternative approach is to specify 'good' candidate structures to use as starting configurations, and this method is used most widely. It requires extensive assumptions about the material investigated, such as which types of atoms are bonded to which and what coordination numbers are present. Calculations have focused on materials that are quite well understood (silicon[2], silica[1,3], common minerals[7]) and the candidate structures used are the observed structures (under a variety of conditions) together with some other well-known arrangements of the correct stoichiometry. This selection of candidate structures can be considered neither rigorous nor exhaustive. Whether or not the minimization process incorporates directional chemical bonds, it is convenient to specify possible structural arrangements by their bond connectivity — the problem is then reduced to the study of periodic networks[8,9]. Major problems include an insufficient understanding of fundamental constraints on the maximum cell size, or on the minimum and maximum allowable densities. For example, the observed distribution of 4-connected three-dimensional zeolite nets falls within a fairly restricted density range[9]. Even incorporating such restrictions, together with additional stereochemical information, the number of stoichiometrically possible networks can be very large. For example, the approximately 500 nets described by Smith[8] could all be used as candidate structures for the silica polymorphs. In general, one needs realistic but more restrictive stereochemical constraints that reduce this number to more manageable proportions. Combinatorial studies of restricted structure types[10,11] show that many potential structures occur even for simple stoichiometries. All of these are likely to correspond to local energy minima, heralding significant problems for an *a priori* energy minimization approach.

Thus a rigorous general solution to the question of crystal-structure prediction may not be forthcoming in the near future. At least, for simple materials, it is possible to deduce accurate metric and physical properties from an approximate atomic arrangement, but much more work is needed in the area of predicting bond connectivities before the problem can even be considered as susceptible to solution.

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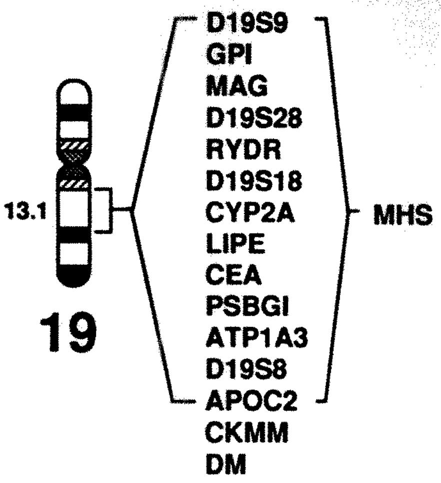
1. Maddox, J. *Nature* **335**, 201 (1988).
2. Cohen, M.L. *Nature* **338**, 291–292 (1989).
3. Tsuneyuki, S., Tsukada, M., Aoki, H. & Matsui, Y. *Phys. Rev. Lett.* **61**, 869–872 (1988).
4. Catti, M. *Phys. Chem. Miner.* **16**, 582–590 (1988).
5. Car, R. & Parrinello, M. *Phys. Rev. Lett.* **55**, 2471–2474 (1985).
6. Martin, T.P. *Angew. Chem.* **25**, 197–296 (1986).
7. Price, G.D., Parker, S.C. & Leslie, M. *Phys. Chem. Miner.* **15**, 181–190 (1987).
8. Smith, J.V. *Chem. Rev.* **88**, 149–182 (1988).
9. Brunner, G.O. & Meier, W.M. *Nature* **337**, 146–147 (1989).
10. Hawthorne, F.C. *Acta Crystallogr.* **A39**, 724–736 (1983).
11. McClarnen, T.J. *J. Solid State Chem.* **26**, 235–244 (1978).

Gene candidate

SIR—The subregional localization of malignant hyperthermia susceptibility (MHS) to chromosome 19q12–13.2 recently reported in *Nature*[1] can be used to suggest or eliminate 'candidate' genes as the site for the primary defect in this disorder. Indeed, MacLennan *et al.*[2] suggest, on the basis of linkage data, that a mutation in the ryanodine-receptor gene probably causes MHS. While awaiting the description of the mutation in MHS individuals which would confirm which gene is responsible for MHS, we would like to suggest another candidate.

It is known that free fatty acid metabolism is abnormal in MHS skeletal muscle: muscle homogenates from humans[3] and isolated mitochondria from pigs[4] indicate that free fatty acid release is greater than two-and-a-half times that of normal controls. These free fatty acids seem to be derived from skeletal muscle triglycerides[4]. Elevated free fatty acid concentrations can increase the release and decrease the uptake of calcium in the sarcoplasmic reticulum[5]. The threshold for calcium-induced calcium release from sarcoplasmic reticulum is also reduced in MHS[6], and fatty acids enhance the release of calcium by lowering this threshold[7]. Free fatty acids are also well known to produce heat by uncoupling oxidative metabolism.

A hormone-sensitive lipase is crucial in mobilizing free fatty acids from stored triglycerides and is regulated by hormonal and neuronal factors[8]. This lipase is activated by catecholamines through cyclic AMP-mediated phosphorylation, and dephosphorylated (inactivated) by insulin[8]. If this insulin inactivation did not occur in MHS individuals, excess free



Regional localization of genes and DNA segments on chromosome 19 (see refs 8–11). Anonymous DNA segments are referred to as 'D' numbers[11]. Gene symbols: GPI, glucose phosphate isomerase; MAG, myelin-associated glycoprotein; ATP1A3, ATPase; Na^+, K^+, α3 polypeptide; RYDR, ryanodine receptor or sarcoplasmic Ca^{2+} release channel; CYP2A, cytochrome P450, subfamily IIA; LIPE, hormone sensitive lipase; CEA, carcinoembryonic antigen; PSBGI, pregnancy-specific β-1-glycoprotein 1; APOC2, apolipoprotein C-II; CKMM, creatine kinase, skeletal muscle; DM, dystrophia myotonia.

fatty acids would be produced and muscle metabolism would be disrupted. Catecholamines are significantly elevated during a hyperthermic crisis and would exacerbate the production of free fatty acids by stimulating the lipase in a potentially catastrophic metabolic spiral.

Complementary DNAs encoding the lipase in the rat have recently been described, and this gene is expressed in skeletal muscle as well as in many other tissues[8]. Mapping data from hybrid cell lines[8,9] assign the human lipase to the light-staining giemsa band 19q13.1, and indicate that this gene is flanked by the same genetic markers as the MHS gene. Therefore, the gene encoding the lipase is at, or near the chromosomal locus for the gene determining MHS (see figure).

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1. McCarthy, T.V. *et al. Nature* **343**, 562–564 (1990).
2. MacLennan, D.H. *et al. Nature* **343**, 559–561 (1990).
3. Fletcher, J.E. *et al. Br. J. Anaesth.* **58**, 1433–1439 (1986).
4. Cheah K.S. *et al. Biochem. biophys. Acta* **638**, 40–49 (1981).
5. Fletcher J.E. *et al. Eur. J. Anesth.* **6**, 355–362 (1989).
6. Nelson, T.E. *J. clin. Invest.* **72**, 862–870 (1983).
7. Fletcher, J.E. *et al. FASEB J.* **4**, A1209 (1990).
8. Holm, C. *et al. Science* **241**, 1503–1506 (1988).
9. Schonk, D. *et al. Cytogenet. Cell Genet.* **51**, 1075 (1989).
10. Schonk D. *et al. Genomics* **4**, 384–396 (1989).
11. *Cytogenet. Cell Genet.* **51(1–4)**, 1–1148 (1989).

Homologue of TFIIS in yeast

SIR—We wish to draw attention to an interesting similarity of amino-acid sequence that we believe has been overlooked. While screening random genomic fragments from the yeast *Saccharomyces cerevisiae* we sequenced[1] a part of the gene *PPR2* which encodes a transcripitonal regulator of dihydroorotase (*URA4*). A search of the NBRF protein databank with the peptide sequence encoded by our clone revealed significant homology with only one other protein — the mouse transcription elongation factor SII (ref. 2). The search did not identify *PPR2* because this sequence was not present in the NBRF database (Release 21·0 (6·89)).

When the sequences of the 128 amino acid and *PPR2* protein and the 301 amino acid SII protein were compared, an overall identity of 40 per cent with respect to *PPR2* and a high degree of conservative amino-acid substitution was revealed (see figure). Strikingly, the two proteins share the same carboxy terminus, having 70 per cent identity in the 44 carboxy-terminal amino acids.

The activity of SII (otherwise called TFIIS[3]) is necessary for efficient RNA polymerase II transcription elongation past template-encoded pause sites, and has been described in mouse[4], *Bombyx mori*[5], bovine species[6], *Drosophila*[7] and HeLa[3] systems. The protein binds to RNA polymerase II in the absence of transcription[3,8]. The SII protein is larger than *PPR2*, but elongation-stimulating activity resides at the carboxy terminus, the very region with homology to *PPR2*.

These observations suggest that *PPR2* functions in the regulation of *URA4* expression at the level of transcriptional elongation, and also that SII may not be a general transcription factor, such as TFIIA,B,D,E and F, but instead, may regulate a specific subset of genes.

The function of the highly conserved carboxy-terminal region is at present unclear, but the absence of any obvious DNA-binding sequence suggests it may contact a component of the transcriptional machinery. A closer analysis of *PPR2* at the molecular level in yeast may be illuminating.

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1. Hubert, J.-C. *et al. EMBO J.* **2**, 2071–2073 (1983).
2. Hirashima, S., Hirai, H., Nakanishi, Y. & Natori, S. *J. biol. Chem.* **263**, 3858–3863 (1988).
3. Reinberg, D. & Roeder, R.G. *J. biol. Chem.* **262**, 3331–3337 (1987).
4. Sekimizu, K., Nakanishi, Y., Mizuno, D & Natori, S. *Biochemistry* **18** 1582–1588 (1979).
5. Hirashima, S., Horikoshi, N., Sekimizu, K. & Natori, S. *Biochem. biophys. Res. Comm.* **160**, 1093–1099 (1989).
6. Rappaport, J., Reinberg, D., Zandomeni, R. & Weinmann, R. *J. biol. Chem.* **262**, 5227–5232 (1987).
7. Sluder, A.E., Greenleaf, A.L. & Price, D.H. *J. biol. Chem.* **264**, 8963–8969 (1989).
8. Horikoshi, M., Sekimizu, K. & Natori, S. *J. biol. Chem.* **259**, 608–611 (1984).

```
PPR2      1 MNKVNNCDTNEAAYKARYRIIYSNVISKNNPDLKHKIANGDITPEFLATCDAKDLAPAPLK
                      :  ||:| |   ||: : :||:|::::  |:|:|:::|  :|:::|:: ||
SII     174 EAIYQEIRNTDMKYKNRVRSRISNLKDAKNPNLRKNVLCGNIPPDLFARMTAEEMASDELK

QKIEEIAKQNLYNAQGATIERSVTDRFTCGKCKEKKVSYYQLQTRSADEPLTTFCTCEACGNRWKFS 12
:: ::::|::: : | |::: : || |||||||:|: :| |:||||||||:||| :|::|||||||
EMRKNLTKEAIREHQMAKTGGTQTDLFTCGKCKKKNCTYTQVQTRSADEPMTTFVVCNECGNRWKFC 301
```

Amino acid alignment of yeast *PPR2* and mouse transcription elongation factor SII(TFIIS). Vertical lines indicate identity, colons conservative replacements.

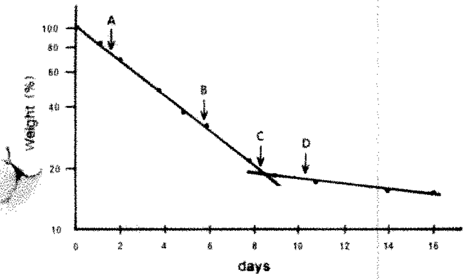
Edible eyeballs from fish

SIR—We report here a simple method of vitrification of denatured proteins such as egg white or vitreous bodies of fish eyeballs which have become opaque by boiling. In vitrification, a material becomes transparent under visible light, and becomes amorphous and rigid like glass.

We vitrified denatured proteins by keeping boiled opaque egg white in a refrigerator after removal of the shell and yolk. As early as a day after refrigeration, the thin, elastic edge began to harden and become transparent. The weight was reduced by 30 per cent after 6 days (see figure), and most of it had hardened and become transparent. When the weight fell to 17 per cent of the original weight, all the egg white was perfectly hard.

The moisture from the boiled egg white evaporated in two stages (see figure). The first-stage evaporation continued until the weight was reduced to 20 per cent of the original, whereupon the second stage began, which continued until all the water in the egg white had evaporated. As these two stages are so different, the moisture lost in the first could be weakly bound to the protein, and in the second stage fairly strongly bound.

Though there have been many experiments concerning bound and free water reported[1–4], our method might reveal something new. There are known to be about 40 proteins in egg white; considering the molecular masses of the main ones[5]



Two stages of moisture evaporation in boiled egg white kept in an ordinary refrigerator and the state of its transparency. Ordinate, weight reduction of boiled egg white. The thin edges of boiled egg white became hard and transparent (vitrified) after the first day in a refrigerator, as shown by arrow A. After 6 days (arrow B) most of the boiled egg white had become hard and transparent. At arrow C (8 days) the evaporation entered the second stage. And finally, after 10 days (arrow D), all the egg white was hard and transparent.

and neglecting the rest we estimated the average molecular mass to be 51,000. The content of the free and bound water was estimated to be 1,900 and 19,000 moles per mole of protein, respectively, in the egg white. In other words, one gram of the protein contained 0.7 gram bound water and 7 gram free water. Assuming that the average molecular mass of amino acids is 110, it can be calculated that one amino acid contains 4 and 40 moles of the bound and free water, respectively.

The vitrified egg white was a plastic pale yellow solid. The plasticity could be due to the increased entanglement of the denatured protein molecules. Vitrified raw egg white, on the other hand, is fine and forms thin fragments. X-ray diffraction of vitrified egg white reveals haloes characteristic of the amorphism seen in common glass, and the pore radius in the protein cluster was 6 angstroms. Neither the egg white nor fish eyeballs become putrified.

In the field of food sciences, it is very common to get dry protein product from its moistured material for preservation, as raw or processed products with high moisture are generally considered to putrify readily. As eggs are so common, it would be unusual and unnecessary to dry boiled eggs for preservation. Vitrification of denatured boiled egg white could therefore have been overlooked as a source of edible protein.

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1. Pauling, L., *J. Am. chem. Soc.* **67**, 555 (1945).
2. Bather, R., Webb, S.J. & Cunningham, T.A. *Nature* **207**, 30 (1965).
3. Love, T.R. *Nature* **211**, 981 (1966).
4. Scheuplein, R.J. & Morgan, L.J. *Nature* **214**, 456 (1967).
5. Bahr, J.W. & Bakst, M.R., in *Reproduction in Farm Animals* (ed. Hafez, E.S.E.) 5th edn. 379 (Lea and Febiger, Philadelphia 1987).

Cautious searches

SIR—Skern *et al.*[1] are correct in stating that the results of computer searches for similarity between proteins must be interpreted with caution, but our paper[2] on the maize bifunctional inhibitor of α-amylase/trypsin merely suggested that both thaumatin and the tobacco pathogenesis-related protein should be re-examined for their possible effects on hydrolytic enzymes due to their strikingly extended sequence homology (52–57 per cent over 200 residues) with the inhibitor. Moreover, the sequence similarities we reported are of much greater statistical significance[3] (Z values of 41.7 and 97.8) than those found by Skern *et al.* for the limited similarity between human rhinovirus 2 and thaumatin (Z=5.8) or trypsin (Z=5.6).

The validity of our suggestion has been supported by several recent discoveries. For example, a potent inhibitor of locust gut α-amylase isolated from seeds of *Coix lachryma-jobi* is, in fact, also an endochitinase[4]. We were prompted to look for the enzyme activity only when a computer search revealed that the inhibitor had a similar sequence to endochitinases. Similarly, when a putative α-amylase/protease inhibitor isolated from barley seeds[5,6] was found to have sequence similarities with phospholipid transfer proteins[7], subsequent assays confirmed that it possessed this function[8]. The further observation that an amylase inhibitor from beans is similar to lectin (agglutinin)-like proteins[9] tends to confirm the belief that computer-aided searches will continue to provide a valuable indication of the possible, but as yet unsuspected or unproven, multifunctional nature of many defensive plant proteins.

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1. Skern, T., Zorn, M., Blaas, D., Kuechler, E. & Sommergruber, W. *Nature* **344**, 26 (1990).
2. Richardson, M., Valdes-Rodriquez, S. & Blanco-Labra, A. *Nature* **327**, 432–434 (1987).
3. Lipmann, D.J. & Pearson, W.R. *Science* **227**, 1435–1441 (1985).
4. Ary, M.B., Richardson, M. & Shewry, P.R. *Biochim. biophys. Acta* **993**, 260–266 (1989).
5. Svensson, B. *et al. Carlsberg Res. Commun.* **51**, 493–500 (1986).
6. Mundy, J. & Rogers, J.C. *Planta* **169**, 51–63 (1986).
7. Bernhard, W.R. & Somerville, C.R. *Archs biochem. Biophys.* **269**, 695–697 (1989).
8. Breu, V. *et al. Calsberg Res. Commun.* **54**, 81–84 (1989).
9. Moreno, J. & Chrispeels, M.J. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7885–7889 (1989).

Killer toxins

SIR—White *et al.*[1] have reported that the yeast killer toxin encoded by the *Kluyveromyces lactis* plasmid pGKL1 is not an inhibitor of adenylyl cyclase, leaving open the question of the mechanism of irreversible growth inhibition in sensitive yeast cells. Recent experience with endochitinase genes from plants and exochitinase genes from bacteria may shed some light on the mode of action of the yeast killer toxin.

Many plants have genes that encode enzymes with chitinase activity; I am most familiar with chitinase-related genes from hybrid poplars[2]. A TFASTA (ref. 3) sequence-similarity search of the GenBank 59.0 DNA (ref. 4) and PIR 21.0 protein (ref. 5) sequence databases using a chitinase-related (Win8) protein sequence (deduced from its complementary DNA sequence) revealed the previously reported global similarity to other plant chitinases and local similarity to plant chitin-binding proteins[2].

Unexpectedly, the large subunit of the yeast killer toxin from pGKL1 (ref. 6) shares a region corresponding to the chitin-binding domain with this collection of plant proteins. When I used the toxin protein itself to search the sequence databases, an additional similarity (outside the domain shared with plant chitin-binding proteins) to the exochitinase from the bacterium *Serratia marcescens* (ref. 7) uncovered was that *Serratia* chitinase has no discernable sequence identity with plant chitinases.

Because fungal cell walls are made predominantly of chitin, and plant and bacterial chitinases are effective antifungal agents[8-10], it seems reasonable to suggest that the large subunit of the *K. lactis* killer toxin arrests the growth of target yeast cells by interfering with cell-wall biosynthesis. It may be useful to test the purified toxin both for the predicted chitin-binding and chitinase activities. If the toxin has exochitinase activity, the domain in common with the chitinase from *Serratia* could be the site of glycosidic bond cleavage.

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1. White, J. H., Butler, A. R. & Stark, M. J. R. *Nature* **341**, 666–668 (1989).
2. Parsons, T. J., Bradshaw, H. D. Jr & Gordon, M. P. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7895–7899 (1989).
3. Pearson, W. R. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2444–2448 (1988).
4. Bilofsky, H. S. *et al. Nucleic Acids Res.* **14**, 1–4 (1986).
5. George, D. G., Barker, W. C. & Hunt, L. T. *Nucleic Acids Res.* **14**, 11–15 (1986).
6. Stark, M. J. R. *et al. Nucleic Acids Res.* **12**, 6011–6030 (1984).
7. Jones, J. D. G. *et al. EMBO J.* **5**, 467–473 (1986).
8. Schlumbaum, A. *et al. Nature* **324**, 365–367 (1986).
9. Mauch, F., Mauch-Mani, B. & Boller, T. *Plant Physiol.* **88**, 936–942 (1988).
10. Roberts, W. K. & Selitrennikoff, C. P. *J. gen. Microbiol.* **134**, 169–176 (1988).

Sensible mathematics

SIR—Dirac[1], after describing the electron self-energy problem and mass renormalization, states, "Most physicists say that QED is a good theory. I must say I am very dissatisfied with the situation because this so-called 'good theory' does involve neglecting infinities which appear in its equations, neglecting them in an arbitrary way. This is just not sensible mathematics. Sensible mathematics involves neglecting a quantity when it is small. . . not just because it is infinite and you do not want it."

In agreement with Dirac, we believe that perturbation theory must be altered or reconstructed into such a form (without spoiling relativistic invariance) that the self-energy integral (in momentum space) taken over the momenta of all virtual photons converges. Feynman, in his paper on QED, multiplied the photon propagator, k^{-2}, by the *ad hoc* factor $-f^2/(k^2-f^2)$, where k is the momentum of the virtual photon (essentially its frequency) and f is an arbitrarily large parameter. Although this convergence factor, which is essentially equivalent to a high-frequency cutoff, preserves relativistic invariance, it is objectionable because of its *ad hoc* character and the presense of f without any theoretical justification. We show below, however, that the Feynman convergence factor appears in the self-energy integral naturally if general relativity is taken into account.

Isham *et al.*[2] have shown that in a 'gravity-modified' QED the "inverse of the gravitational constant appears as an effective cut-off mass" in the calculation of the electron self-energy, but their calculation is so encumbered by formalism that it is difficult to see the physics that is involved. For that reason, our calculation below, which leads essentially to the same result and to a general relativistic Feynman convergence factor, is important. Our calculation takes into account the gravitational reddening suffered by a virtual photon owing to the increased mass of the emitting particle produced by its recoil momentum.

Because momentum, unlike energy, is conserved in each single virtual process (each integral for a single virtual photon is taken over all configuration space, so that there is no uncertainty in momentum, as emphasized by Dirac[1]) the frequency ν_0 of the virtual photon, just when it is emitted, is given by the equation $2\pi\hbar\nu_0/c = m_0v/[1-(v/c)^2]^{1/2}$; here m_0 is the resting mass of the emitting particle, v is its recoil velocity, and $m = m_0/[1-(v/c)^2]^{1/2}$ is its effective mass, which redshifts the frequency of the virtual photon in accordance with the Einstein formula $\nu = \nu_0[1-(2Gm/c^2r)]^{1/2}$, where r is the Compton wavelength $\hbar/m\,c$ of the emitting particle. From our first equation (the conservation of momentum), we have for the recoil velocity

$$v = (h\,\nu_0/m_0c)/[1+(h\,\nu_0/m_0c^2)^2]^{1/2}$$

and

$$m = m_0\,[1+(2\pi\hbar\nu_0/m_0c^2)^2]^{1/2}$$

On substituting this into the equation for the effective frequency of the virtual photon, we obtain

$$\nu = \nu_0[(1-\frac{2Gm_0^2}{\hbar c}) - 8\pi^2G\hbar\ \nu_0^2/c^5]^{1/2}$$

Because the second term $2Gm_0^2/\hbar c$ in the parenthesis is negligible for the electron, we can exclude it and write $\nu = (f^2)^{-1/2}(f^2-\nu_0^2)^{1/2}\nu_0$, where $f^2 = c^5/8\pi^2G\hbar$, a universal constant (the square of a frequency), is of the order of 10^{95} and therefore extremely large, as required by Feynman. Multiplying f by h and dividing by c^2, we obtain the universal mass $m = (\hbar c/G)^{1/2}$ as the cut-off mass. This is just the Planck mass, which indicates that gravity is important in perturbation theory.

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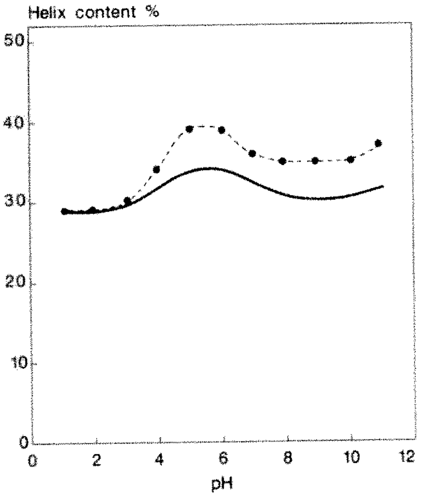
2. Isham, C. J., Salam, A. & Strathdee, J. *Phys. Rev.* D**3**, 1805 (1971).

Short alpha-helix stability

SIR—Because C-peptides, the 13-residue peptides simulating an α-helix of RNase, are significantly helical in aqueous solution near 0 °C[1,2], the helicity has been attributed to specific side-chain interaction. The helicity is 30 times more than that predicted by Zimm–Bragg theory with host–guest stability parameters[3].

Yet the observed helicity of C-peptides (between 15 per cent and 45 per cent depending on amino-acid composition and pH) is close to that expected for uncharged polylysine fragments of the same length, where specific side-chain interactions (salt bridges, hydrogen-bonding, aromatic interactions) are absent.

By direct measurement[4], the helix-stability parameters for uncharged polylysine have been found to be $s_\alpha = 1.3$ (at about 0 °C) and $\sigma_\alpha = 0.0025$. Using these values, the Zimm–Bragg theory[5] gives 30 per cent helicity for 13-residue polylysine with –CONH groups at either end (as in C-peptides). Thus the 'general level' of the observed helicity of C-peptides is normal (at about 0 °C) for a short peptide consisting of helix-forming residues without the intervention of specific side-chain interactions.



pH dependence of α-helical content for the C-peptide (acetyl-AETAAAKALRAHA-amide) at 3 °C in 0.1 M NaCl. Solid line, experiment[2]; dotted line, theory[7]. The temperature change of s_α was calculated using the helix–coil transition enthalpy ΔH = −1.1 kcal mol^{-1} (see ref. 4) for all amino acids. All other parameters are independent of temperature[6,7].

At room temperature, the helicity of polylysine fragments must be half as much; s_α falls[4] to 1.14 at 25 °C, similar to the decrease of helicity found for the C-peptides[1]. It is relevant that helicity of about 5 per cent has been predicted[6] for the corresponding fragment of unfolded RNase.

The helicity of a peptide differs from the 'general level' because of a multitude of specific side-chain interactions, (usually rather weak), many of which (usually very weak) have been taken account of theoretically[6,7]. Thus the 1.2-fold increase of helicity of C-peptides on ionization (see figure) is explained by the electrostatic attraction of Glu_2^- and His_2^+ to the partial charges at the nearest termini of a fluctuating α-helix. Present theory[6,7] may thus serve as a guide for singling out the more specific effects observed in experiments with short peptides[2].

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1. Shoemaker, K.R., Kim, P.S., York, E.Y., Stewart, J.M. & Baldwin, R.L. *Nature* **326**, 563-567 (1987).
2. Shoemaker, K. R. *et al. Cold Spring Harbor Symp. Quant. Biol.* **LII**, 391-398 (1987).
3. Sueki, M. *et al. Macromolecules* **17**, 148-153 (1984).
4. Barkaya, T.V. & Ptitsyn, O. B. *Biopolymers* **10**, 2181-2197 (1971).
5. Flory, P.J. *Statistical Mechanics of Chain Molecules* chapter 7.5 (Wiley, New York, 1969).
6. Finkelstein, A.V. & Ptitsyn, O. B. *J. molec. Biol.* **103** 15-24 (1976).
7. Finkelstein, A.V. *Program ALB* Brookhaven Protein Data Bank and EMBL (1983).

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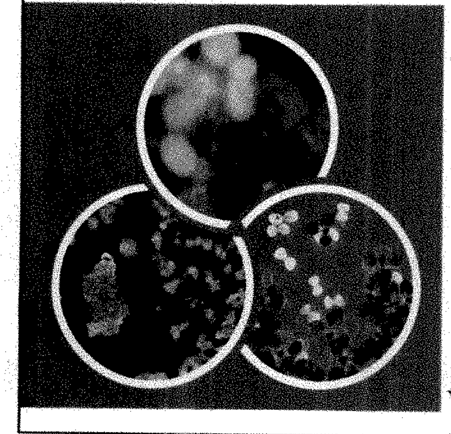

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Translation from the Greek

V. Reynolds

Oedipus in Evolution. By Christopher Badcock. *Basil Blackwell: 1990. Pp. 221. £29.95, $37.95.*

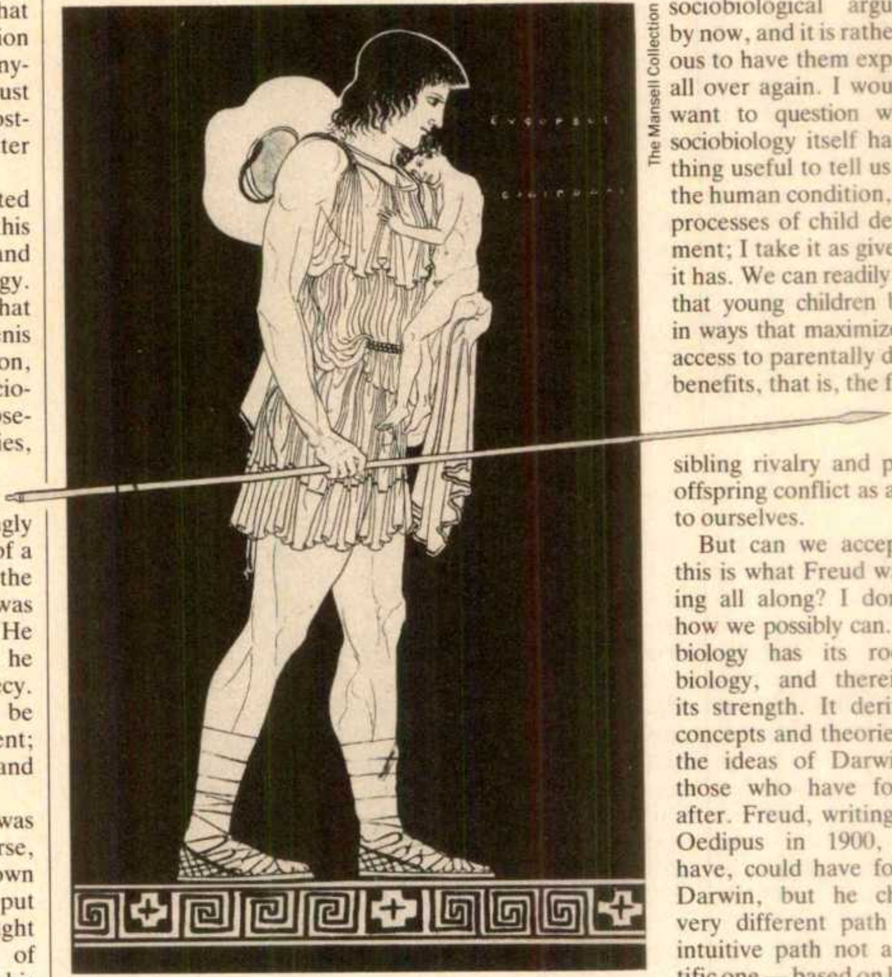
I ASSUME most readers of *Nature* feel they can get on quite well without Freud. Perhaps, indeed, most people can. Yet we are told incessantly by media pundits and even some academics and "talking heads" that our whole way of looking at ourselves this century is freudian, that Freud has changed us. So the question arises whether modern science has anything to say about this, whether we must just accept that we are somehow post-freudian, or whether we can do better than that.

Badcock's self-appointed task, started in earlier volumes and continued in this one, is to show that Freud was right, and to show this by recourse to sociobiology. His technique is to demonstrate that things like the Oedipus complex, penis envy, castration complex or regression, are Freud's way of describing what sociobiologists like Robert Trivers have subsequently shown to be evolved propensities, the outcome of natural selection.

Who was Oedipus? A character in a Sophoclean play who unwittingly killed the King his father, as a result of a prophecy, where three roads met, on the way from Thebes to Delphi. What was Oedipus thinking of, to kill his father? He wasn't thinking, he didn't even know; he was driven, driven by fate, by prophecy. Link number one with evolution; to be driven, not to be a wholly free agent; evolution has two drivers, survival and reproductive success.

Oedipus later found out that it was indeed his father he had killed and, worse, that he had gone on to marry his own mother. So deep was his shame that he put out his eyes. In addition, he had brought famine and despair to the people of Thebes, and blinding himself expiated his sin. This is Greek tragedy at its finest. Badcock's preoccupation with sociobiological reinterpretation must pale by comparison, and indeed so must Freud's own work, though that at least has elements of the bizarre about it.

Freud took the sophoclean story and made of it an interpretation of an aspect of childish behaviour, the longing of the young boy to possess his mother exclusively and to oust his father from the family. Each of us, said Freud, and Badcock accepts this without question, goes through this phase, after the earlier oral one. Having emerged from a total preoccupation with sucking the mother's nipple, boys start to get sexually possessive, and mothers respond warmly to their offspring's amorous advances. Why should these things (shocking to Freud's generation, boring to ours) go on? Because, says Badcock, young children have been selected during evolution to behave in ways that deter the mother from investing in another child, so as to maintain the maximum amount of parental investment for themselves.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The infant Oedipus rescued from the mountain by a shepherd, where Apollo had warned that Oedipus would kill his father.

In Freud's equation, Oedipus is you and me; we are driven to (want to) kill our father and to (want to) marry (that is, possess) our mother. The twin pillars of the equation are: unwitting = unconscious and driven = evolved. The unconscious human psyche has evolved in such a way that now, during childhood, as it develops, it sets itself these two unattainable goals — patricide and incest. Does sociobiology really support this? According to Badcock it does; Freud has been vindicated by sociobiology.

> I am arguing that Oedipal behaviour in little boys . . . should be looked at from the point of view of its costs and benefits to the individual child in terms of parental investment. According to this way of looking at things, Oedipal behaviour would constitute a kind of "rehearsal" or "advertisement" of the boy's potential reproductive success, aimed at securing preferential parental investment because of the potentially much greater reproductive success which an individual male can have in the context of a polygamous mating system. (Page 87.)

(Polygamy is here used to include officially monogamous systems in which there is extramarital sex.) We know the sociobiological arguments by now, and it is rather tedious to have them explained all over again. I would not want to question whether sociobiology itself has anything useful to tell us about the human condition, or the processes of child development; I take it as given that it has. We can readily accept that young children behave in ways that maximize their access to parentally derived benefits, that is, the facts of sibling rivalry and parent–offspring conflict as applied to ourselves.

But can we accept that this is what Freud was saying all along? I don't see how we possibly can. Sociobiology has its roots in biology, and therein lies its strength. It derives its concepts and theories from the ideas of Darwin and those who have followed after. Freud, writing about Oedipus in 1900, might have, could have followed Darwin, but he chose a very different path — an intuitive path not a scientific one — based on his own idiosyncratic interpretations of people's dreams and neuroses. Freud thus has to be classed with those thinkers who eschewed the scientific tradition, not with those who embraced it. Today, Freud is history. If some of his insights seem akin to the findings of sociobiology, he is not thereby vindicated, other than in the sense that one or two of his hunches were near the mark. Badcock demonstrates commendable loyalty to the old man, but I fear that there is nothing anyone can do to put him back on his pedestal. ☐

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State of the art

David Bailin

Electroweak Interactions. An Introduction to the Physics of Quarks and Leptons.
By Peter Renton. *Cambridge University Press: 1990. Pp. 596. £60; $110.*

QUANTUM electrodynamics (QED) is a relativistic quantum field theory formulated by Feynman, Dyson and Schwinger around forty years ago. Its incredible success in describing and predicting purely electromagnetic processes with apparently limitless accuracy in terms of very few parameters, naturally suggested that the other interactions of particle physics, the weak and strong interactions, should also be described by relativistic quantum field theories. We now know that this expectation is correct, and that the weak and electromagnetic interactions are described by a unified gauge field theory called electroweak theory, while the strong interactions are described by quantum chromodynamics (QCD).

Electroweak theory differs from QED in several important respects. First, the gauge symmetry group ($SU(2) \times U(1)$) underlying the theory is larger than the simple $U(1)$ group (of transformations on a circle) which underlies QED. Second, this group is non-abelian, meaning that it is non-commutative. Finally, the theory is 'chiral', reflecting the fact that weak interactions, unlike electromagnetic ones, are not invariant under the parity transformation: we can say that left and right chiral states have different weak charges. None of these differences is much more than technical, and in principle an $SU(2) \times U(1)$ invariant gauge theory could have been formulated at any time after about 1954. The really vital difference from QED is that the symmetry is broken. If it were not, the weak gauge particles, the W and Z bosons, would be massless, just as the photon is and, because the theory is chiral, the electron, muon and other fermions would also be massless. Breaking the gauge symmetry is even easier than preserving it, but in general the breaking destroys the 'renormalizability' of the symmetric theory. In a renormalizable theory, such as QED, the theory is rendered finite by absorbing (ultraviolet) divergences into a finite number of (unpredicted) parameters. The problem with breaking the symmetry by hand, as it were, is that an infinite number of such renormalizations is needed, and the theory loses the ability to predict, which is the hallmark of its paradigm (QED). The trick, we now know, is to break the symmetry 'spontaneously'; the dynamics preserves the symmetry, but the ground state (the particle-physics vacuum) breaks it. (It was Weinberg's use of this technique on Glashow's gauge symmetric lagrangian which finally led to the electroweak quantum field theory, whose renormalizability was demonstrated in the early 1970s.)

Most of the components of this theory have now been tested against experiment with overwhelming success. It is the renormalizability of the theory which permitted the prediction of the W and Z boson masses to within about 1 GeV c^{-2} of the values measured when the particles were triumphantly discovered at CERN in 1983. Similarly, the theory now provides upper and lower limits on the top quark mass before its discovery. The final 'proof' that the symmetry is spontaneously broken must await the discovery of the Higgs boson, whose mass is unfortunately not predicted by the theory. The undeniable success of the theory naturally requires that graduate students of particle physics learn the theory and how it works. This demands not merely the study of quantum field theory, and QED in particular, but also the novel features of electroweak theory to which I have alluded. Increasingly it falls to experimentalists to perform the theoretical calculations against which the data are tested, and which 15 years ago were the preserve of theoreticians.

It is therefore entirely appropriate that this book on electroweak theory is by an experimentalist, in fact one who is currently engaged in precision tests of the theory at the DELPHI detector on LEP, the electron–positron collider opened at CERN six months ago. Having an experimentalist as its author, the book is naturally replete with an abundance of very impressive data detailing just how successful the model has been. The problem for the author of such a book is where to begin, and Renton goes right to the very beginning, dealing with wave equations, field operators and QED, including its renormalization, in the first third of the book. All these topics are of course well covered (in more detail) in many books, some more than 30 years old. In petroleum retailing it is well known that if a crossroads (say) has three filling stations, then this demonstrates that the location is ideal for selling petrol and that a fourth filling station will also thrive. As it is in gas stations, so it is apparently in bookselling. Renton declares in his preface that "the main emphasis is on the understanding of physical processes in terms of lowest order calculations". He stresses that higher order calculations are important and refers the reader to "more advanced texts" for details. I think this is a pity. The renormalization is rather complicated, as the novel features of electroweak theory require techniques which are not required in QED. Nevertheless, as the whole point of the theory is that it is renormalizable, and therefore admits precision tests, and since experimentalists are beginning to do these calculations, I think that some attempt at covering this topic should have been made, probably dispensing with the earlier material. Another theorist's gripe would be the failure to cover the 'chiral anomalies'. It is precisely the requirement of 'anomaly cancellation' which makes us so certain that the top quark must exist. Not many books discuss either of these, so perhaps my (academic's) criticism can be rebutted by the (retailer's) response that the market is not yet ready for such an innovation.

In any case, my overall judgement is very positive, and I think that the book will be well received, especially by experimentalists. It is about much more than electroweak interactions. (It includes QCD, and the possibilities beyond the standard model.) In many ways the subtitle conveys more accurately than the main title what is in the book: an (excellent) account of the current state of particle physics. □

David Bailin is in the School of Mathematical and Physical Sciences, University of Sussex, Brighton BN1 9QH, UK.

Culture club

R. H. A. Coutts

Genetic Engineering of Crop Plants.
Edited by G. W. Lycett and D. Grierson. *Butterworths: 1990. Pp.293. £65.*

IN the first chapter of this collection of papers presented at the 49th Nottingham Easter School in Agricultural Sciences, Edward Cocking illustrates the importance and the impact of plant cell and tissue culture on plant genetic engineering; most of the subsequent contributions serve only to reinforce this statement. In some cases, where researchers are dealing with plants amenable to tissue culture and which can be regenerated, such as tobacco and potato, the results are exciting. But with other, more recalcitrant species, such as some monocotyledonous plants and legumes, optimism abounds.

The scope of the book is certainly wide ranging, from coat protein-mediated protection against plant virus infection to nodulation genes of *Rhizobium*. In fact these chapters, respectively from the laboratories of Roger Beachy and Tony Johnston, are highpoints in the book: the assembly of useful data includes not only that obtained by the named researchers efforts but reviews that of others. The underlying enthusiasm of molecular biologists, especially through the discovery of transgenic plant technology, is evident in the crisp and stylish presentation.

▶

What sets this book aside from the usual collection of conference proceedings is that the contributors not only present their own recent data but have tried to integrate their thoughts on other research in their sphere of interest. Such presentations were particularly evident in the contribution from the Durham plant molecular biology group.

I am unsure as to whether the absence of methodology sections from the chapters will appeal to the purists, but good reference lists at the end of most chapters should encourage the curious. The book is generally well-illustrated with good pictorial evidence of results, particularly in the presentations by Stanford *et al.*, on wound-induced genes in potato, and Covey *et al.*, on pathogenesis of cauliflower mosaic virus.

One undeniable problem of publishing the proceedings of any scientific meeting is the inevitable time difference between presentation and publication. Nevertheless, the editors have assembled a lucid and informative volume within a year, which is to be applauded. Even so, some of the contributions have or are about to appear in other volumes, which detracts a little from this one. Also, the competitive nature of the science may have deterred some, but not all, of the participants from divulging new information — again, an unavoidable problem. To those in the business of teaching the genetic engineering of crop plants, the synthesis of complex data and a volume of research papers into succinct accounts of exciting science will be extremely useful.

It has become *de rigeur* to publish the proceedings of international conferences almost as an afterthought. Because of this fact many researchers' and lecturers' shelves are weighed down by an ever increasing number of books which rapidly lose their scientific value, either because they are out of date and do not reflect current scientific dogma or they are badly written and poorly presented. It is a pleasant change to read a book which does not have these pitfalls, and I hope that it is well read and well used by students and researchers alike. Indeed, I feel it is these areas of higher education, especially at the undergraduate and first-year postgraduate stages, where the book will find its most enthusiastic reception. ☐

R. H. A. Coutts is in the Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, UK.

Correction

Information supplied by the Science Photo Library for the picture accompanying the review of *Science-based Dating in Archaeology* (*Nature* **345**, 126; 10 May 1990) incorrectly stated that the Oxford radiocarbon accelerator could date samples up to 100,000 years in age. This should have read 40,000 years. ☐

Class act

J. Z. Young

Cephalopodes. Edited by Katharina Mangold. *Traité de Zoologie. P-P. Grassé. Tome V, Fasc. 4. Masson: 1989. Pp.804. FF1,100. (In French.)*

OCTOPUSES, squids and cuttlefishes are among the most interesting inhabitants of the seas, both scientifically and economically. Moreover, the relics of their ancestors in the rocks are the most familiar of all fossils. This excellent book about them is the latest edition to Grassé's monumental treatise, now extending to 39 tomes and covering the whole animal kingdom.

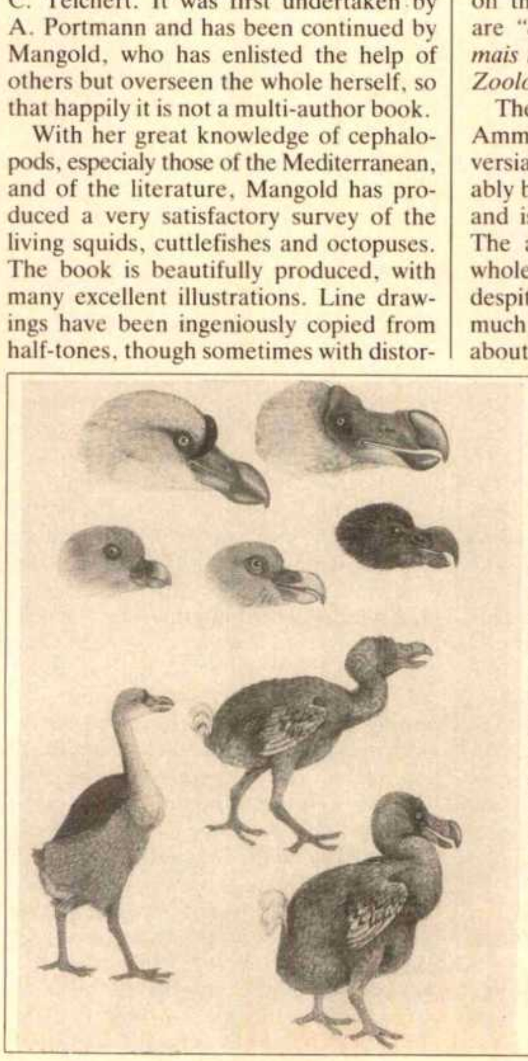
Anyone writing a treatise on Cephalopods has to please two distinct populations of readers — zoologists and palaeontologists. These groups have different aims, use different languages and even have different views of scientific method. Yet the work of each group requires knowledge of the results of the other. The volume under review is really two books, a long one by zoologists, led by K. Mangold, and a shorter, by the palaeontologist C. Teichert. It was first undertaken by A. Portmann and has been continued by Mangold, who has enlisted the help of others but overseen the whole herself, so that happily it is not a multi-author book.

With her great knowledge of cephalopods, especialy those of the Mediterranean, and of the literature, Mangold has produced a very satisfactory survey of the living squids, cuttlefishes and octopuses. The book is beautifully produced, with many excellent illustrations. Line drawings have been ingeniously copied from half-tones, though sometimes with distortion of the cell pattern; for instance in nervous tissue. There are full bibliographies and a useful index.

This book has been in preparation for many years. The zoological part has been kept up to date, carrying references up to 1987. The palaeontology section, however, goes only to 1983. But the point of such a work is not that it includes the latest news or fresh original data, but that it provides a thorough view of the many aspects of the animals. Besides description of the structure and functioning of the various organ systems and their embryology, there are valuable ethological chapters on distribution, migrations, parasites and predators, including fishermen (but curiously little about prey). The systematic section briefly reviews every genus and will be valuable in itself.

Cephalopods provide the material for an increasing volume of biological research, ranging from molecular biology to neurophysiology. Workers who are studying, say, the pigments of blood or the membranes of giant nerve fibres can learn in this book the place of their tissues in the life of the octopus or squid. They may not find much detail about their own topic: for instance a footnote warns that works on the giant nerve fibres and synapses are "*extrêment nombreux et importants, mais ils dépassent le cadre d'un Traité de Zoologie*".

The section on the fossil Nautiloids and Ammonoids compresses a huge controversial subject into 70 pages. It will probably be too summary for palaeontologists and is rather hard going for zoologists. The author discusses the origin of the whole class and of the various groups, but despite the vast number of fossils there is much controversy and few conclusions about the causes of their evolution and



These drawings of the dodo *Didus ineptus* (top, middle and lower right), the Solitaire *Pezophaps solitaria* (top and bottom left), and the mainland Green Pigeon, Sao Tome Green Pigeon and Tooth-billed Dove (three heads in the middle), are by Jonathan Kingdon, taken from his recent book *Island Africa*. Full of beautiful ilustrations by the author, the book documents the evolution of Africa's rare animals and plants. It is a record of the treasuries of species to be found in enclaves throughout the continent. Published by Collins, price £25.

extinctions. The function of the shell is largely to regulate buoyancy and it is a pity that more has not been deduced from its various shapes about the mode of life of the animals that lived in them, and the reasons for their success or failure.

Teichert discusses the eight 'crises' in which the ammonoids nearly became extinct before they finally disappeared at the end of the Cretaceous. The crises were very irregular, occurring at intervals varying from 5–120 million years, curiously not affecting the nautiloids at the same times. The author considers and rejects various hypotheses put forward to explain this, considering that multiple causes were at work and that most authors have been "*trop simpliste*" in their discussions.

One of the uses of a treatise such as this is to provide classifications, and it is unfortunate that those given in the two parts of the book do not agree. Nor is there any clear view of the evolutionary relationship of the various groups. It is particularly disappointing that in neither part is there any discussion of the relationship of the various orders or families of modern cephalopods — admittedly a difficult subject. The difficulty partly results from the shocking scarcity of data about genetics within the group. The authors are not to blame for this. They have produced a splendid book, which will be useful for many years. ☐

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Radical thought

Asa Briggs

The Invention of Progress. The Victorians and the Past. By Peter J. Bowler. *Basil Blackwell: 1989. Pp 231. £25, $34.95.*

MUCH, though not all, of the voluminous writing on "the Darwinian revolution" fails to do justice to other nineteenth-century intellectual developments that enable the revolution to be placed in perspective. In linking developments in biology to developments in geology, anthropology, archaeology and, not least, history and pre-history, Peter Bowler is not perhaps as original as he claims. Yet he has written a comprehensive, stimulating and ambitious book which starts not with biology but with history and which moves on via "the fascination of the past" and through sociology, philology, theology (less fully treated, though often touched upon) and law before turning to "the antiquity of man", "missing links", "the origin of races", "fossils and progress", "the ascent of life", "the record of the rocks" and "progress by leaps". The attractive title "The *Invention* of Progress", backed by well-chosen illustrations as well as text, points to the author's unifying explanation of what was written and said in a remarkably impressive range of often heated victorian debates. "The idea of progress was imposed upon history to create the sense of order that the Victorians craved. In a very real sense they *invented* the past, since all the factual discoveries of antiquaries and archaeologists had to be interpreted within a conceptual scheme that satisfied the cultural demands of the age." "Only by looking across the whole field of Victorian historical studies", Bowler argues, can the content and implications of the different but related victorian debates be fully understood.

Bowler's account of many of the particular debates makes fascinating reading even when the reader is familiar with the content of the debate in question. He is a clear expositor and knows well how to summarize different points of view and how to chart the way in which the points of view of particular individuals changed. Yet it is easier for readers asked for the first time to consider the whole range of the debates to take some of the arguments more seriously than others. The author's approach is that of an intellectual, not a social historian, and he does not always tell us how seriously or how widely some of the debates influenced contemporaries. Moreover, he does not discuss how far in a period of increasing, if still at the end of the period imperfect, academic specialization there were people who were interested in all the topics focused upon. Is he assembling material that was not assembled at the time? His own sense of purpose in trying to relate everything to everything else is at least as determined and as powerful as that sense of "cosmic teleology" which he imputes to most of the writers with whom he is concerned.

In treating progress as the key to everything, Bowler is at pains to insist that there was no unanimity about its character, and in relating progress to history and science he is himself inevitably leaving out economics and technology, subjects that generated other kinds of debate about the nature of progress. For his own purposes he finds it necessary to distinguish only between two nineteenth-century approaches to "progress" — the first sequential or continuous; the second episodic or cyclical. The latter, according to him, for long predominated. Thus, the belief that each "race" — and he is especially illuminating on this difficult subject — had contributed to "the ascent of man", only to give way before "higher types", was paralleled in the writings of "palaeologists opposed to gradual evolution". In each case, Bowler seems to be arguing, the reasons why the episodic or cyclic view was for a long period the dominant view lay outside intellectual history. The view was significant because it made it possible for victorians either to continue to believe in "a divine plan" or in "imperial mission": "Like the great empires of the past, each of the vertebrate classes had had its period of glory before being displaced by the next highest manifestation of the Creator's power."

In the last few densely packed pages of the book, which deal with the decline of the idea of progress in the late-nineteenth century and twentieth-century — "degeneration", cultural relativism, the "new psychology", "Spenglerism" — Bowler argues also that it was factors outside intellectual history as much as changes in the content of the sciences that accounted for the change. "It is difficult to believe that the belated recognition of the full potential of Darwin's theory in the early-twentieth century played more than a minor role in the crisis of confidence that eventually destroyed the Victorian concept of progress."

It should be added that *The Invention of Progress* incorporates a thesis which the author has already advanced in an earlier study, *The Non-Darwinian Revolution*, published in 1988, a thesis with two conclusions. First, while *The Origin of Species* played a major "catalytic role in converting the world to evolutionism", late-nineteenth century evolutionism remained "emphatically developmental in character" and "natural selection remained peripheral to most biologists' views on the mechanism of evolution until well into the twentieth century". "The open-ended undirected character of evolution in Darwin's original theory was ignored or misrepresented". Second, before Darwin there was "an active movement by political radicals and younger medical men to exploit the materialist implications of evolutionism in their campaign against the political, academic and medical establishments, and Darwin himself was "unable or unwilling" to "sever the link between evolutionism and the developmental way of thinking". "Radical materialism" was, in consequence, held back.

Both parts of the thesis are stimulating. The second, however, raises questions of the individual motivation of scholars — and others — which require further discussion. "Victorian values", which figure in Bowler's index, themselves remain matters of debate. So, too, does "materialism", "radical" or otherwise. ☐

Asa Briggs is Provost of Worcester College, Oxford OX1 2HB, UK.

- Also recently published, *In Pursuit of a Scientific Culture* by P. A. Dale, documents the search of victorian writers for a philosophical replacement for romanticism. Published by University of Wisconsin Press. Price, hbk $42.50; pbk $17.50.

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Evolution and detectability of comet clouds during post-main-sequence stellar evolution

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Stars leaving the main sequence enter a luminous phase, during which circumstellar comet disks would be partially evaporated. Such a process could explain some observations of water masers and complex molecules in envelopes of giant and supergiant stars, and might provide a means of searching for Oort-type clouds—and by inference, past planetary formation—around other stars.

IN some 4–5 × 10^9 years the Sun will exhaust its hydrogen supply and begin its post-main-sequence evolution, a common process for lower main-sequence stars[1]. During post-main-sequence evolution, late-type stars typically attain luminosities between $6\times10^3\,L_\odot$ and $6\times10^4\,L_\odot$ for periods of one million to several tens of million years. We develop a time-dependent model for the destruction of comets within the sublimation radius of typical post-main-sequence stars, and show that the effects of such a luminosity increase on the star's reservoir of comets will be dramatic. We also point out that comet-cloud destruction produces several detectable signatures. If comet clouds are common around solar-type stars, then prodigious cometary destruction should be an observable aspect of post-main-sequence stellar evolution.

We begin by evaluating the radiative equilibrium temperature T_e of a unit surface normal to the post-main-sequence insolation at astrocentric distance R

$$T_e(R)=\left[\frac{(1-A)L_{\mathrm{poms}}}{4\pi\varepsilon\sigma R^2}\right]^{1/4} \qquad (1)$$

where σ is the Stefan–Boltzmann constant, A is the surface bolometric Bond albedo, ε is the comet surface emissivity, and L_{poms} is the luminosity of the post-main-sequence star. Using representative parameters of $A=0.04$, $\varepsilon=0.9$ and $L_{\mathrm{poms}}=6\times10^3\,L_\odot$ (2.3×10^{37} erg s^{-1}), one finds

$$T_e(R)=3{,}530\,R_{\mathrm{AU}}^{-1/2}\left(\frac{L_{\mathrm{poms}}}{6\times10^3\,L_\odot}\right)^{1/4}\ \mathrm{K} \qquad (2)$$

where R_{AU} is the comet's astrocentric distance measured in AU. Equation (2) predicts that vigorous water sublimation will occur at distances up to ~400 AU around a typical post-main-sequence star. More limited water sublimation will proceed at distances as great as ~600 AU; CO and other extremely volatile ices will sublimate at distances as great as 10^4 AU.

The presence of a vast cloud of comets orbiting the Sun at large heliocentric distances is now well established[2,3]. This assemblage of water-rich objects is believed to consist of three components. The innermost component of the Sun's comet cloud is the Kuiper (or Kuiper–Duncan) disk, a belt- or disk-like ensemble of 10^7–10^9 cometary objects with an unknown amount of smaller debris. The Kuiper disk is believed[4] to be the reservoir of the short-period comets, to lie between thirty and a few hundred AU from the Sun, and to have formed essentially *in situ* during the epoch of outer Solar System planetary formation. Various workers[5–7] have demonstrated the presence of particle disks at similar distances around other stars, but the presence of macroscopic objects (such as comets) in these disks has not been established. At a distance of ~1,000–20,000 AU from the Sun is the inner Oort cloud, a larger, more flared disk believed to consist of some 10^{12}–10^{13} comets ejected from the planetary region during the accumulation of the giant planets. The disk-like nature of the Kuiper Disk and inner Oort cloud result from their association with the planetary Solar System; dynamical (for instance, stellar) perturbations are insufficient to convert these inner disks into spheres on timescales comparable to the Sun's age. Finally, beyond the inner cloud is an isotropic outer Oort cloud containing ~10^{11}–10^{12} comets extending as far as 2×10^5 AU from the Sun; the outer cloud is unstable to external perturbations and is resupplied from the inner cloud by the perturbing influence of passing stars and molecular clouds.

The thermal conditions described by equation (2) suggest that comets and other objects in the Kuiper disk will suffer intense mass loss during the Sun's post-main-sequence phase. In the more distant Oort cloud, comets on approximately circular orbits will be subject to very low water sublimation rates, but will experience some loss of volatile species including CO_2, CO, CN, Ar, N_2, NH_3 and CH_4 from their outer layers. As we will show, Oort cloud comets on eccentric orbits penetrating the sublimation region will suffer repeated, intense episodes of water sublimation and mass loss.

Sublimation of the comet cloud

When a solar-type star exhausts its hydrogen supply and ends hydrogen-core burning, its core contracts and hydrogen-shell burning begins. This causes the luminosity to steadily increase to a value of ~300 $L_\odot$ over ~10^8 years as the star becomes a red giant. At the end of this period, the water sublimation radius will have increased from 2.5 AU ($L=L_\odot$) to ~40 AU ($L=300\,L_\odot$). This initial slow luminosity increase will have drastic

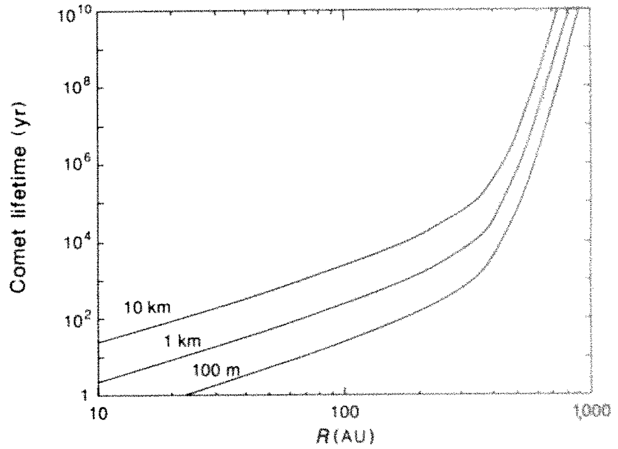

FIG. 1 Sublimation-destruction curves (equation (5)) for water-ice bodies of radius 100 m, 1 km and 10 km as a function of astrocentric distance R for $L_{\mathrm{poms}}=6\times10^3\,L_\odot$ and assuming a density of 0.5 g cm^{-3}.

and potentially observable influences on any planets and their satellites (a subject beyond the scope of this work). Because the volume of the sublimation zone increases in proportion to $L^{3/2}$, the number of active comets should increase dramatically.

Following complete hydrogen exhaustion and core contraction, the star will rise along the giant branch of the H–R (colour-luminosity) diagram, terminating in the helium flash. Although the flash luminosity may exceed $10^5\ L_\odot$, the duration of the flash is too short (hours to days) to sublimate a large fraction of the cometary reservoir. The star then becomes a horizontal-branch star. For a period of $\sim 10^7$ years, low-mass post-main-sequence stars obtain a luminosity of several thousand $L_\odot$. During this period the water sublimation radius will extend into the region of the Kuiper disk and the inner Oort cloud.

To model the survival time of a comet disk against post-main-sequence heating, we conservatively assume that comets consist of only water ice and refractory dust. To calculate the water sublimation rate from a spherical icy body, we simultaneously solve for the global average surface temperature, including the effects of latent heat release

$$T(R) = \left[\frac{(1-A)L_{\text{poms}}/(16\pi R^2) - H(T)\dot{m}(T)}{\varepsilon\sigma}\right]^{1/4} \qquad (3a)$$

where the sublimation mass loss rate per unit surface area is

$$\dot{m}(T) = P_s(T)\sqrt{\frac{\mu}{2\pi kT}} \quad \text{g cm}^{-2}\,\text{s}^{-1} \qquad (3b)$$

Here $H(T)$ is the specific heat of sublimation, $P_s(T)$ is the vapour pressure of water ice at temperature T, μ is the relative molecular mass of the water in grammes, and k is Boltzmann's constant. We use the relation for the saturation vapour pressure of water ice given by Lebofsky[8]

$$P_s(T) = 10^{-2{,}403.4/T + 9.183837}\ \text{torr} \qquad (4)$$

where the temperature is specified in kelvin. Given a numerical solution to the mass loss rate, the lifetime of a spherical comet of radius r_c and density ρ at astrocentric distance R under constant stellar insolation is just

$$t_c(R, r_c) = \frac{4 r_c \rho}{\dot{m}(R)} \qquad (5)$$

Figure 1 depicts the sublimation timescale for water-ice objects, as a function of astrocentric distance. These curves were calculated according to the sublimation model given above by numerically shrinking test objects until they sublimated to destruction. Comets smaller than 10 km in radius and within 100 AU of such a star will be sublimated to destruction within $\sim 10^3$ years after a solar-type star becomes a luminous red supergiant. By the end of the post-main-sequence phase ($\sim 10^7$ yr), sublimation will have slowly destroyed the comets and other volatile-rich debris trapped inside a few hundred AU of the parent star.

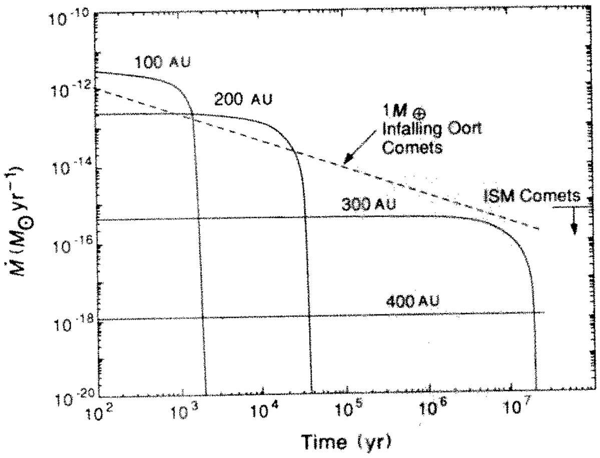

FIG. 2 Mass loss rate and survival time of a cometary disk around a 6,000-$L_\odot$ star as a function of astrocentric distance. Each solid curve represents the mass loss rate from a 100-AU-wide annulus of the disk centred on the labelled distance. Dashed line gives the total (that is, distance integrated) mass loss rate from infalling comets derived from an Earth-mass (1 $M_\oplus$) Oort cloud. The horizontal bar on the right gives an upper limit for infalling interstellar comets.

In addition to the destruction of comets trapped on orbits entirely within the sublimation zone, infalling Oort cloud comets that pass through the sublimation zone will also lose mass. Models of the Oort cloud indicate that moderate orbital eccentricities are common, so this source of mass loss may be significant. For example, integrating $\dot{m}$ from equation (3b) over an orbit with a semi-major axis a of 1,200 AU and eccentricity e of 0.80, one finds that a 10-km comet consisting of 50% water ice by mass and having a 3% surface albedo will sublimate to destruction in 10^7 years around a $6\times10^3\ L_\odot$ post-main-sequence star. On core helium exhaustion, solar-type stars form a planetary nebula and a white dwarf, which then fades slowly in luminosity.

We now explore the time-dependent evolution of comet disks and Oort-like clouds in the presence of a luminous post-main-sequence star. The solid lines in Fig. 2 depict the results of a sublimation rate calculation of mass loss in the Kuiper disk. An assemblage of comets of 5-km radius with total mass 1 $M_\oplus$ were placed in a disk with a flat radial mass distribution extending from 100 to 1,000 AU. All objects were assumed to lie in nearly circular orbits in the disk. Each solid line in Fig. 2 represents the mass loss from those comets in a zone 100 AU wide centred on the labelled distance. The mass loss rates are for water ice; dust or other materials liberated by water sublimation will suffer loss rates in direct proportion to their relative abundance to water. In this model, each comet is diminished in size as it loses mass until it sublimates to destruction. Comet mantling effects were not included in the model. A constant $L_{\text{poms}} = 6\times10^3\ L_\odot$ was assumed; higher luminosity results in a larger sublimation destruction zone, faster destruction, and increased mass loss rates at all distances. In a population of objects with various sizes, the smallest objects will be lost first; the instantaneous mass loss rate of the disk itself is not, however, set by the object sizes but by stellar luminosity and the total projected surface area of all objects in the disk.

Figure 2 shows that in the first $\sim 5\times10^4$ years after the post-main-sequence phase begins, sublimation will cause all comets trapped within 200 AU to sublimate to destruction. Beyond 300 AU, temperatures are sufficiently low that comets are not destroyed, even over 10^7 years. Thereafter, ignoring stellar pulsations, the loss rate of material from disk comets will consist of: (1) the partial destruction of those comets trapped within a few hundred AU of the star; (2) the sublimation of infalling comets that penetrate the water sublimation radius on orbits from the Oort-type cloud; and (3) the sublimation of any interstellar comets that pass through the sublimation zone of the star. The water mass loss rate, $\dot{M}_{H_2O}$, owing to the second mechanism (dashed line) was calculated for an orbit-averaged loss rate of a population of comets distributed uniformly in semi-major axis and eccentricity, as

$$\dot{M}_{H_2O} = \sum_a \sum_e N_c(a, e)\left(\frac{1}{T_{\text{orb}}}\right) \times \int_0^{T_{\text{orb}}} \pi r_c^2\, \dot{m}_i(t, a, e, L_{\text{poms}})\, dt \qquad (6)$$

Here $N_c(a, e)$ is the number of comets with orbital semi-major axis a and eccentricity e, and $\dot{m}_i(t, a, e, L_{\mathrm{poms}})$ is the water-ice mass loss rate per unit surface area at time t in the orbit. T_{orb} is the orbital period for comets with semi-major axis a, circling a star of mass M and constant luminosity of 6,000 $L_\odot$. For the case depicted in Fig. 2, comets of 5-km radius and total mass 1 $M_\oplus$ were distributed uniformly in 400 (a, e) orbit bins in the cloud.

For the case depicted (a 1 $M_\oplus$ of comets in the disk and equal mass in the outer Oort cloud, infalling comets dominate the mass loss after ~5×10^4 years. The mass loss rate is proportional to the initial mass supply; therefore, if the mass of Oort cloud comets exceeds the mass of Kuiper disk comets, then the infalling Oort cloud comets can dominate the mass loss rate at earlier and earlier epochs. If the comet cloud has total mass of ~1 Jupiter-mass, then the dashed line would be raised by a factor of ~300 over what is shown.

An upper limit to the mass loss rate from interstellar comets passing through the sublimation zone on hyperbolic orbits was determined by calculating the sublimation rate for an interstellar comet population based on the upper limit[9] on the space density of interstellar comets; the depicted result assumes a uniform distribution of impact parameters. We find that the expected mass loss from interstellar comets is small compared with that from the Kuiper disk and infalling comet cloud sources.

Comparing the total cometary mass loss over the post-main-sequence phase to the initial mass of the cometary reservoir, we found that although a substantial fraction (such as 30%) of the mass of a Kuiper-like disk can be entirely destroyed by post-main-sequence sublimation, only a small fraction (typically ~1%) of the mass of the infalling Oort-like reservoir will be lost. Most objects in the halo are protected by their large periastron distances.

Because Oort clouds and comet disks are optically thin, the destruction timescale for any given object in the cloud is only a function of its albedo, mass and astrocentric distance, and of the parent star's post-main-sequence luminosity. Smaller objects are destroyed faster. The time at which the mass loss curves in Fig. 2 turn down is set by the point at which the disk mass supply is exhausted at that distance. Therefore, the mass loss rate is supply-limited, rather than energy-limited. Further, although the disk mass loss rate at each distance depends on the total surface area exposed to insolation, the time at which the mass supply is exhausted is independent of the number of objects in the disk.

Cometary water in post-main-sequence outflows

The destruction of orbiting comets and debris provides a natural explanation for the water in, and several other attributes of, many post-main-sequence stellar outflows. These include the ubiquitous H_2O and OH emission surrounding post-main-sequence stars[10,11], the presence of HCN, organics and sulphur-bearing molecules around Miras and M supergiants[12] and the coexistence of both ice grains and water at distances of several hundred AU in the outflows of red giants. Indeed, the water mass loss rates for many Mira and OH/IR star outflows[11,13] are comparable to the range of sublimation rates predicted by our comet-cloud sublimation model.

The historical explanation for the production of water in stellar outflows is photochemistry[14,15]. In these models, the OH abundance in circumstellar envelopes is controlled by exchange reactions and the dissociation of H_2O by interstellar ultraviolet radiation. The reaction $OH + H_2 \leftrightarrow H_2O + H + 0.69$ eV converts OH into H_2O in the warm inner envelope, whereas ultraviolet photodissociation produces OH in the outer envelope. These processes can explain OH densities ~$1\ \mathrm{cm}^{-3}$ at distances of 10^{16} cm from the star[14], which are comparable to those found in our models of Jupiter-mass Oort clouds during the first several hundred thousand years of post-main-sequence evolution (Fig. 3*b*). We believe that the comet-cloud sublimation model may provide a more natural, and in some respects more comprehensive, explanation for the OH and H_2O outflows.

We stress that the comet sublimation model does not predict the mechanism for H_2O or OH photon emission, only the ultimate source of the water. Presumably, the OH is created through dissociative kinetic and photon reactions of the cometary water vapour. Although post-main-sequence sublimation of the comet cloud can produce water mass loss rates comparable to those observed in the outflows of many Mira and OH/IR stars, we also point out that comet-cloud sublimation can account neither for the bulk stellar mass outflow rate nor for most of the dust in the outflow. That is, the sublimation mechanism is not a substitute for intrinsic stellar mass loss or for the production of dust in cool stellar atmospheres.

To demonstrate the inability of comet-cloud sublimation to provide the observed dust, we calculate the optical depth of a spherical dust outflow from the parent star owing to cometary sublimation. The dust : ice mass ratio in comets is ~1 : 1 so it is reasonable to assume that the dust and water production rates are similar. The dust optical depth as a function of wavelength λ can be written

$$\tau_\lambda \approx 10^{-3}\left(\frac{\dot{M}_c}{10^{-12}\ M_\odot\ \mathrm{yr}^{-1}}\right)\left(\frac{10\ \mathrm{km\ s}^{-1}}{v_w}\right) \times\left(\frac{100\ \mathrm{AU}}{R_s}\right)\left(\frac{0.2\ \mu\mathrm{m}}{\lambda}\right)^{1.5}\left(\frac{0.1\ \mu\mathrm{m}}{\bar{r}_g}\right) \quad (7)$$

where $\dot{M}_c$ is the dust mass loss rate assuming a dust: ice ratio of 1:1. Here v_w is the velocity of the stellar wind, R_s is the length scale of the sublimating region, and $\bar{r}_g$ is the number-weighted mean radius of the dust grains. Equation (7) assumes spherical cometary grains with $\rho = 0.2\ \mathrm{g\ cm}^{-3}$ and an infrared emissivity of $(0.2\ \mu\mathrm{m}/\lambda)^{1.5}$. Using the $\dot{M}_c$ calculations shown in Fig. 2, equation (7) demonstrates that if an Earth-mass cometary disk sublimates, very low optical depths are to be expected. Compared with the nominal optical depths of OH/IR and Mira

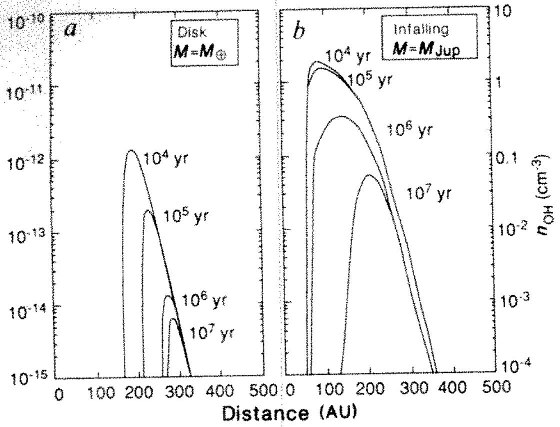

FIG. 3 *a* and *b* depict the mass loss rate of a Kuiper-like disk and a distant Oort-like cloud, respectively, around a 6,000-$L_\odot$ star. Each solid curve represents the mass loss rate at the indicated time after the stellar luminosity is turned on. The strong dependence of sublimation rate on temperature produces an annular 'sublimation ring'. Variations in the stellar luminosity will cause the time evolution of the ring to be more complicated. The effect of infalling Oort cloud comets is to broaden the ring inward and prevent the peak mass loss rate from decaying below some level set by the orbital distribution and total mass of the infalling comets.

stars[10], such optical depths are almost negligible.

Returning to water sublimation, an important distinguishing characteristic of a sublimating comet disk is its annular geometry. Such an annulus arises from the fact that water sublimation rates are extremely sensitive to temperature, and are therefore steep functions of the astrocentric distance. Figure 3*a* depicts the time evolution of such an annular ring (or torus) for a constant post-main-sequence luminosity of $6\times10^3\ L_\odot$. Figure 3*b* depicts the mass-loss evolution from an infalling Oort-like halo of comets. The model for comet-disk sublimation produces geometric structures similar to actual very-long-baseline interferometry (VLBI) observations of some post-main-sequence stars. These observations[13,16–18] show water and OH emission in smoothly expanding, thin 'shell-like' or 'ring-like' regions near the sublimation distance of 70–1,000 AU from the parent star (depending on the stellar luminosity). Although both the classical photochemical models and the comet-cloud sublimation model can explain shell-like haloes of water vapour, photochemical models cannot easily explain the annular geometries[13,19]. Such geometries would naturally result from the sublimation of assemblages similar to the planar Kuiper disk.

The connection between cometary sublimation and the H_2O/OH outflows observed around M giants, Miras and OH/IR stars can be validated through future tests. For example, in the case of the ring-like emission regions, the sublimation model predicts that peak H_2O and OH number densities will lie inside the sublimation radius and near the rotational plane of the parent star. Furthermore, for both disk destruction and infall of Oort-type clouds, the model predicts an inverse correlation between the post-main-sequence age of the star and the water production rate, and a positive correlation between stellar luminosity and water mass loss rate. The model also predicts a phase-coherent correlation between the abundances of OH and H_2O and fluctuations in the bolometric luminosity of the parent star. One expects a phase delay between sublimation and stellar luminosity pulsations to be set by the time of light travel from the star to the location of the water source. In the complicated near region, such a phase delay will be short; at the far region, up to a distance of several hundred AU, the delay should be of the order of hours to days.

Finally, we suggest that the complicated, time-varying OH- and water-maser structures within 10–100 AU of many post-main-sequence stars, called 'blobs'[13,16,19,20], may actually represent individual orbiting bodies on eccentric trajectories. We note that H_2O and OH masers have been observed in cometary atmospheres[21]. According to stellar-atmosphere models, the post-main-sequence emission blobs are difficult to explain systematically and would not be expected to be in orbit around their parent stars. Although these emission blobs (or 'hotspots') have been observed to change position with time, no systematic study of their movements has been made. To test the comet-cloud hypothesis, we therefore suggest that these emission features should be observed (and tracked) on a timescale that is short compared with nominal keplerian timescales around Mira and OH/IR stars. By using both the high plane-of-sky spatial resolution and high line-of-sight velocity resolution inherent to VLBI, it should be possible to determine if the movements of these objects can indeed be fit to bound keplerian orbits, as would be expected for comets. We point out that if the blobs actually move in keplerian orbits, then orbital inversion also offers the promise of accurately determining the masses of numerous single Miras and other post-main-sequence stars.

Summary and outlook

We have modelled the destruction of volatile-rich comet disks and Oort-type clouds around luminous post-main-sequence stars. Such models are in agreement with several aspects of existing observations of water and complex molecules in the envelopes of giant and supergiant stars. We suggest that these attributes result from the destruction of orbiting comet clouds. If confirmed, our results would establish the common existence of Oort-type clouds around other stars and would constitute indirect evidence for sites of past planetary formation. Struck-Marcell[22] has conducted a study of the fate of planets and planetary satellites surrounding post-main-sequence stars. As shown there, high-luminosity phases of post-main-sequence stellar evolution can also have dramatic effects on planets.

We are now constructing more-detailed models of comet-cloud destruction and its observable signatures using numerical, time-dependent evolutionary tracks of post-main-sequence stellar evolution. In a future paper we will present these results and compare the applicability of the comet-destruction model to observations of specific well-known Miras and OH/IR stars.

For now, we have shown that luminous post-main-sequence stars will destroy resident volatile cometary-debris disks within several hundred to a thousand AU of the parent star on timescales shorter than the stellar post-main-sequence lifetime. Such a fate will remove the Solar System's Kuiper comet disk. Furthermore, comets on plunging orbits from the Oort cloud will repeatedly enter the post-main-sequence sublimation zone and lose substantial mass. The relative mass loss rates from the disk and infalling comet sources depend on the relative masses and orbital distributions of the disk and Oort cloud, and on the stellar luminosity. It is difficult to imagine a process that would quench comet-cloud sublimation around a luminous post-main-sequence star. Therefore, if volatile-rich bodies are orbiting such stars, high water production rates would naturally result.

The expected water loss rates from comet disks of several Earth masses or greater are similar to the observed mass loss rates around many post-main-sequence stars. Several other observable attributes of sublimating comet clouds are consistent with observations of many Mira and OH/IR stars. The Mira and OH/IR states are thought to be normal phases in the post-main-sequence evolution of late-type stars.

The importance of the detection of comets around stars is that comet formation strongly suggests planetary formation. Several observable signatures from sublimating comet clouds should be seen around red giants, Miras and other post-main-sequence stars possessing comet disks or clouds. We suggest that several already-observed attributes of Mira and OH/IR stars may be related to comet sublimation.

If comets are indeed responsible for commonly observed features in post-main-sequence stellar environments, then the probability of comet formation (and by extension, planetary systems) around single main-sequence stars is likely to be high. □

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1. Iben, I. & Renzini, R. *Phys. Rep.* **105**, 329–367 (1984).
2. Hills, J. G. *Astr. J.* **86**, 1730–1740 (1981).
3. Bailey, M. E., Clube, S. V. M. & Napier, W. M. *Vistas Astr.* **29**, 53–180 (1986).
4. Duncan, M., Quinn, T. & Tremaine, S. *Astrophys. J.* **328**, L69–L74 (1988).
5. Aumann, H. H. *et al. Astrophys. J.* **278**, L23–L28 (1984).
6. Harper, D. A., Loewenstein, R. F. & Davidson, J. A. *Astrophys. J.* **285**, 808–812 (1984).
7. Backman, D. & Gillett, F. C. in *Cool Stars, Stellar Systems and the Sun* (eds Linsky, J. L. & Stencel, R. E.) 340–350 (Springer, New York, 1987).
8. Lebofsky, L. A. *Icarus* **25**, 205–217 (1975).
9. Sekanina, Z. *Icarus* **27**, 123–133 (1976).
10. Jones, T. J., Hyland, A. R., Wood, P. R. & Gatley, I. *Astrophys. J.* **273**, 669–680 (1983).
11. Bowers, P. F. & Hagen, W. *Astrophys. J.* **285**, 637–647 (1984).
12. Nejad, L. A. M. & Millar, T. J. *Mon. Not. R. astr. Soc.* **230**, 79–86 (1989).
13. Bowers, P. F., Johnston, K. J. & de Vegt, C. *Astrophys. J.* **340**, 479–502 (1989).
14. Goldreich, P. & Scoville, N. *Astrophys. J.* **205**, 144–154 (1976).
15. Netzer, N. & Knapp, G. R. *Astrophys. J.* **323**, 734–748 (1987).
16. Reid, M. J., Muhleman, D. O., Moran, J. M., Johnston, K. J. & Schwartz, P. R. *Astrophys. J.* **214**, 60–77 (1977).
17. Jura, M. & Morris, M. *Astrophys. J.* **292**, 487–493 (1985).
18. Herman, J. & Habing, H. J. *Astr. Astrophys. Suppl.* **59**, 523–555 (1985).
19. Johnston, K. J., Spencer, J. H. & Bowers, P. F. *Astrophys. J.* **290**, 660–670 (1985).
20. Lane, A. P., Johnston, K. J., Bowers, P. F., Spencer, J. H. & Diamond, P. J. *Astrophys. J.* **323**, 756–765 (1987).
21. Crovosier, J. *Astr. J.* **90**, 670–674 (1985).
22. Struck-Marcell, C. *Astrophys. J.* **330**, 986–991 (1988).

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Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis

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Crystals of Ha-Ras p21 with caged GTP at the active site have been used to investigate the conformational changes of p21 on GTP hydrolysis. The structure of the short-lived p21 · GTP complex was determined by Laue diffraction methods. After GTP hydrolysis, substantial structural changes occur in the parts of the molecule implicated in the interaction with GTPase-activating protein. The trigger for this process seems to be a change in coordination of the active-site Mg^{2+} ion as a result of loss of the γ-phosphate of GTP.

THE gene products (p21 proteins) of the *ras* oncogene family are thought to be involved in a signal transmission pathway similar to those involving G proteins[1-3]. Analogous with the latter systems, and with other GTP-binding proteins such as the polypeptide elongation factors, p21 seems to be in an 'active' state when complexed with GTP, and in an 'inactive' state when complexed with GDP, the product of the p21 GTPase activity. It is this reaction, or more exactly its activation by GTPase-activating protein (GAP), that is modified in transforming mutants of Ha-Ras p21 (refs 4-8). Thus, an understanding of the GTP hydrolysis mechanism, the accompanying protein structural changes and the way in which these are modified by—and modify—the interaction with GAP are crucial for clarifying the molecular basis of oncogenic transformation by mutant p21 proteins.

We have recently shown that the Ha-*ras* oncogene product p21 can be crystallized with a photolabile GTP derivative (caged GTP; Fig. 1) at its active site, and that after photolytic removal of the protecting group, hydrolysis of GTP to GDP occurs in the crystal at the same rate as with p21 in solution[9]. We describe here the use of such crystals to provide information on the structural changes accompanying GTP hydrolysis in this system. Whereas the structures of static states of the system could be determined using classical (monochromatic) crystallographic methods, the short lifetime of the p21 · GTP state made it necessary to use the polychromatic Laue diffraction method (for reviews, see refs 10, 11).

Experimental strategy

The structural data reported refer to the guanine-binding domain of p21 (amino acids 1-166, that is, without the C-terminal 23 residues), which is easily crystallized[12,13]. The approach used is summarized in Fig. 1. The structures of stable or quasi-stable complexes in this scheme were determined by monochromatic X-ray diffraction methods. This was not possible for the relatively short-lived complex between cellular p21 (p21c) and GTP. This structure was determined by the Laue method using synchrotron radiation ($\lambda = 0.7$–2.0 Å) after cleavage of the 2-nitrophenylethyl group in crystals of p21c · caged GTP, and before significant hydrolysis of GTP to GDP had occurred. The structure of the protein was also determined (in the same crystal) by the Laue method, 14 min after initiating the GTPase reaction by removal of the protecting group, that is, during GTP hydrolysis (Fig. 2).

Description of the structures

(1) General. The overall structures of the complexes analysed here, p21 · caged GTP, p21 · GTP and p21 · GDP, are the same in terms of the general organization of their secondary structural elements (α-helices, β-sheets) and also identical to that reported for the p21 · GPPNP complex (see below)[13,34].

(2) p21 · GTP. One of the main reasons for determining the p21 · GTP structure was to compare it with the structure of p21 complexed to GPPNP, a non-hydrolysable GTP-analogue[13,34]. The interactions around the site of modification of the analogue, the β-γ bridging atom, are important for judging the relevance of the latter structure and for justifying higher resolution studies.

A data set of p21 was obtained under Laue diffraction conditions during the first 5 min after photolysis of the crystalline p21 · caged GTP complex (Fig. 2). We expected that the principal species at the active site would be GTP. This is confirmed by the electron density map (Fig. 3*b*) which shows the nucleotide bound to the active site in a manner analogous to GPPNP and with three clearly discernible phosphate peaks. No electron density was detected that could be attributed to the cage group. The base-protein interactions that have been identified in the p21 · GPPNP structure are present, as are the interactions of the protein with the phosphate groups[13,34]. Figure 4 demonstrates the similarity between the structures of the p21 · GPPNP and p21 · GTP complexes, as exemplified in the glycine-rich phosphate-binding loop. There is a hydrogen bond between the amide hydrogen of Gly 13 and the β-γ bridging oxygen of GTP or the β-γ bridging nitrogen of GPPNP, in the respective structures. Because the energy of the hydrogen bond to the nitrogen in GPPNP is expected to be less than that to the oxygen in GTP, this may explain the weaker binding of GPPNP to p21 (by a factor of ~10). This interaction may also explain why replacement of the bridging oxygen by groups which cannot form hydrogen bonds (CH_2, CF_2; ref. 12 and unpublished observations) leads to an even greater reduction of affinity than replacement by the NH group.

The Mg^{2+} ion is coordinated to the nucleotide by the γ- and β-phosphate groups (pro-R oxygen) and to the protein by the

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TABLE 1 Cell parameters and data statistics for p21 · nucleotide complexes

Nucleotides	p21c Cell axis (Å) $a=b$	p21c Cell axis (Å) c	p21(G12V) Cell axis (Å) $a=b$	p21(G12V) Cell axis (Å) c	p21c Reflections (total)	p21(G12V) Reflections (total)	p21c Reflections (unique)	p21(G12V) Reflections (unique)	p21c R_{sym}†	p21(G12V) R_{sym}†	p21c Resolution (Å)	p21(G12V) Resolution (Å)
Caged GTP	41.0	164.8	41.0	177.7	7,290	8,143	2,703	3,551	12.8	8.2	3.0	2.8
GTP	41.1	164.8	40.5	161.3	**	11,868	**	5,411	**	4.7	**	2.6
GTP/GDP*	41.1	164.0	—	—	**	—	**	—	**	—	**	—
GDP	40.4	156.5	40.7	156.0	8,172	12,440	2,731	4,201	7.7	7.1	2.8	2.8

The large change in c-axis on GTP hydrolysis probably arises from the change in the structure of the effector region (Fig. 5). **, See Fig. 2 legend.

* Laue data set obtained 14 min after photolysis.

† $R_{sym}\Sigma_h\Sigma_i|(I_{h,i}-I_h)|/\Sigma_h\Sigma_i I_{h,i}$, where $I_{h,i}$ are the intensities of symmetry related reflections occurring uniquely.

functional groups of Ser 17 and Thr 35. In the 1.35-Å structure of p21 · GPPNP, two molecules of water are also coordinated to the metal ion, one of them providing a bridge to the carboxyl group of Asp 57 (ref. 34). Only the latter has been identified in the p21 · GTP structure. But as Mg^{2+} is normally hexacoordinated, it is likely that there is a water molecule in this position that has not been identified.

As in the p21 · GPPNP complex, the ε-amino group of Lys 16 interacts with an oxygen of the γ-phosphate group (not the one that is a ligand to Mg^{2+}), and also, more weakly, with the β-phosphate group. The latter interaction was not mentioned explicitly in ref. 13, but is present on re-inspection of the 2.5-Å structure of p21 · GPPNP and is conspicuous in the 1.35-Å structure. So, the β- and γ-phosphate groups are doubly coordinated with Mg^{2+} and the NH_3^+ group of Lys 16.

As in the p21 · GPPNP complex, the residues in the loop L4 (amino acids 59–65) seem to be able to adopt several conformations. In particular, one position of the carbamoyl group of Gln 61 is close to a water molecule (Wat 175 in the p21 · GPPNP structure[34]) which itself is close to the γ-phosphate. This water molecule is believed to be involved in the GTPase reaction[34]. In contrast to the p21 · GPPNP structure, the density of the first residues of the following α-helix in the p21 · GTP structure is not well defined.

Recent evidence suggests that there should be a structural difference between the p21 · GTP complex described here and the p21 · GPPNP complex described previously[13,34]. An isomerization reaction of the p21 · GTP complex has been identified and this seems to be rate-controlling in the overall GTPase reaction[14]. As this step also occurs with GPPNP, it follows that the p21 · GPPNP complex should correspond to the structure of p21 · GTP immediately before the hydrolysis step. But the p21 · GTP complex examined here using the Laue method should correspond to the complex before the conformational change that leads to production of the hydrolytically competent state, as it is assumed to hydrolyse GTP too rapidly for it to accumulate. Similar experiments to those described here, but using caged GPPNP, should lead to a detailed characterization of this isomerization. The structural change must be subtle, as it has not so far been identified by comparison of the p21 · GPPNP and p21 · GTP structures. But it may occur in the region of amino acids 61–68 (loop L4 and the start of α-helix α2), which cannot be compared because of bad density in the p21 · GTP structure.

(3) p21 · GDP. The structure of p21c · GDP (arising from hydrolysis of GTP after its photolytic release from caged GTP) was determined by crystallography using monochromatic radiation. There are several differences at the active site when compared with the GTP state, which are directly or indirectly due to the loss of the γ-phosphate group. Lys 16 still interacts with the backbone carbonyl group of residues 10 and 11, but not with the β-phosphate. The distance between the amino group of Lys 16 and the oxygens of the β-phosphate group is now too large for the interaction seen in the p21 · GTP and p21 · GPPNP states to occur. The coordination of Mg^{2+} is similar to that seen for p21 · GTP or p21 · GPPNP. But there is only one interaction with the nucleotide (β-phosphate group). The interaction with Asp 57 is now direct, as it is with the corresponding aspartate residue (Asp 80) in the structure of the EF-Tu · GDP complex[15,16] and in the p21 · GDP complex[17], not through a water molecule as in the triphosphate structures. Also, the Mg^{2+} to Thr 35 distance has increased to a value that is too great for direct interaction (4.6 Å, compared with 2.4 Å in the p21 · GTP state).

In contrast to the p21 · GTP structure, there is good density for loop L4. This differs from the report of very weak electron density for residues 61–65 (ref. 17). The diameter of loop L4 has increased compared with the p21 · GTP structure and the structure in this region is now well defined.

There are large differences between the structures of the effector-binding region (residues 32–40) in the p21 · GTP and p21 · GDP states (discussed below).

(4) The effector-binding region. The most dramatic differences between the p21 · GDP structure and that of p21 · GTP or

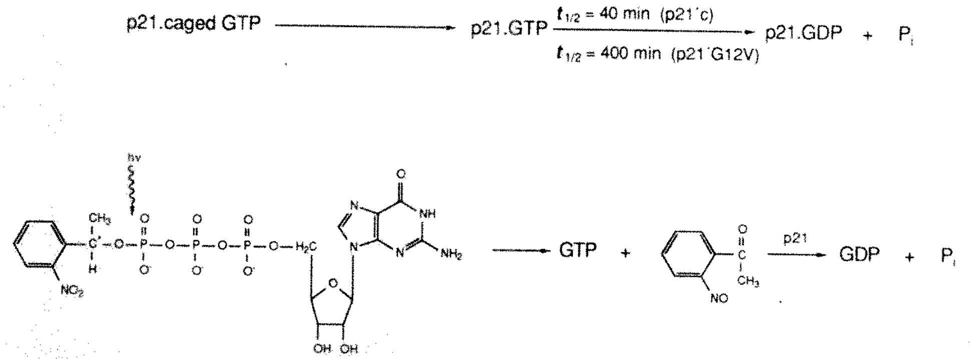


FIG. 1 Experimental scheme. Flow diagram for the generation of p21 · GTP and p21 · GDP complexes. p21c, Cellular (wild-type) protein; p21(G12V), oncogenic mutant in which glycine 12 is replaced by valine. Crystals of p21 · caged GTP (ref. 9) were used to generate the GTP and GDP complexes of p21 by photolytic removal of the protecting 2-nitrophenylethyl group and subsequent hydrolysis. The structures of p21 complexed with caged GTP or GDP were determined by monochromatic X-ray diffraction methods, as was that of the GTP complex of p21(G12V) which was kept stable during the measurement by cooling to 4 °C. This was not possible for the relatively short-lived complex between p21c and GTP. This structure was determined by the Laue method (Fig. 2). Crystals of p21 · GDP (nucleotide content verified by HPLC) were obtained after photolysing p21 · caged GTP crystals and waiting for the GTPase reaction to take place. For practical reasons, X-ray diffraction data were collected 19 h after photolysis. Monochromatic X-ray data were collected and processed as described[13]. The unit cell parameters and data statistics of the different p21 · nucleotide complexes are summarized in Table 1.

p21 · GPPNP are seen in this region (main differences between residues 32–36). This is the part of the molecule that is thought to interact with GAP (as shown by mutational analysis; ref. 18); it is therefore of interest to observe this structural change. Tyr 32 and Pro 34 have flipped to the other side of the loop, as has Asp 33 (albeit in the opposite direction) (Fig. 5). Tyr 32 now seems to interact more strongly with the pyrrolidone ring of Pro 34 than it does in the GTP structure. We cannot yet provide a detailed causal explanation for this structural change but it seems likely that the conformational change is associated with, and is perhaps due to, a change in the position of the metal ion. The interaction of Mg^{2+} with the (now missing) γ-phosphate group seems to be replaced in the p21 · GDP structure by a direct interaction with the carboxyl group of Asp 57 (in p21 · GTP and p21 · GPPNP, this interaction is indirect through a water molecule). Thus, the double positive charge on the Mg^{2+} ion, that is neutralized in the triphosphate structures by interaction with two phosphate groups, is now neutralized by one phosphate group and one carboxyl group. In addition, as mentioned above, Thr 35 is no longer directly coordinated to Mg^{2+} in the p21 · GDP state. The same structural changes are observed in the transforming mutant protein p21(G12V) (Gly 12 → Val). As this is known to interact with GAP in the triphosphate state, and not in the GDP-bound state, it is not surprising that the same structural change is observed on hydrolysis of GTP.

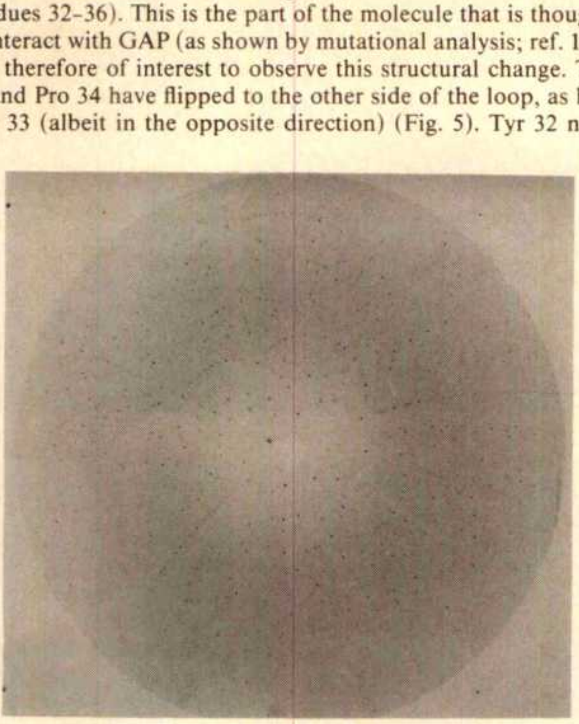


FIG. 2 Laue-Photograph of p21c · GTP obtained 4 min after photolysis. Crystals of p21 · caged GTP (ref. 9; space group $P\ 3_2\ 2\ 1$, $a=b=41.0$ Å, $c=164.8$ Å, size ~0.4 × 0.4 × 0.2 mm) were mounted completely surrounded by artificial mother liquor by either wedging in tapered quartz capillaries or by immobilizing between pipe cleaner fibres. Only freshly prepared artificial mother liquor containing Tris buffer, $MgCl_2$, polyethylene glycol 400 and 40–50 mM DTE was used. Laue diffraction data were collected on film (one film pack consisting of six pieces of CEA reflex 25 film) at X31 located in Hamburg/DESY/HASYLAB during parasitic user time. For this Laue experiment, the white beam was focused by an eightfold segmented gold mirror with 1:1 focus, to obtain sufficient flux at the specimen. Absorption limited the long wavelength maximum to ~2.0 Å, whereas the short wavelength limit was 0.6–0.7 Å because the critical angle for reflection from the mirror was exceeded. The crystal was at random orientation relative to the X-ray beam, and the crystal–film distance was 11.8 cm. The angle of the spindle was tilted by 7° and exposures (10–15 s) of three different orientations of the crystal differing by 15° were collected for one data set. Only 2 out of 26 crystals of wild-type p21 were ordered sufficiently for Laue data analysis. Photolysis was achieved using a Xenon flash lamp[9]. Ten flashes, of ~1 min total, were used to obtain quantitative conversion to GTP (ref. 9). Laue data sets were collected from the same crystal 4 and 14 min after photolytic removal of the protecting group. A third data set 20 min after photolysis showed severe radiation damage (crystal was in the white beam for ~2 min) and thus was discarded. Films were scanned on an Optronics P-1000 HS rotation drum microdensitometer with a 50-μm raster size and an optical density range of 0–2. Data were processed as described previously[29,30] using a suite of programs (GENLAUE, INTLAUE, AFSCALE, LAUENORM) developed at Daresbury[31]. No wavelength deconvolution was used. There were no significant difficulties processing the Laue data collected in Hamburg with the programs optimized for the experimental conditions in Daresbury. During the refinement of the crystal orientation and the unit cell parameters (using the program GENLAUE; ref. 31) the crystal-to-film distance was fixed until the end of refinement. We discarded any questionable spots and therefore chose generous spot-to-spot distance constraints (1.0 mm) and, owing to the elongated diffraction spot shape (spot diameter was set to 0.8 mm in GENLAUE), a large box size (1.2 mm). The wavelength range used for scaling of the reflexes on the six films in one film-pack (AFSCALE; ref. 31) was 0.7–2.2 Å. This program also applies the obliquity and Lorentz-polarization correction. Only reflections with intensity $(I) > 1.5 \times \sigma(I)$ were retained for further processing. Finally, a wavelength-dependent normalization was performed with LAUENORM (ref. 31) by comparing Friedel equivalent reflections measured at different wavelengths. Wavelength curve fitting was performed with two separate curves to eliminate discontinuity associated with the absorption edge of bromine (0.97 Å) in the photographic films. As the additional gold absorption edge (due to coating of the mirrors) in the intensity versus wavelength-dependence coincides with the bromine absorption edge, it was not necessary to use more complicated wavelength normalization procedures. The best *R*-factor was obtained using a wavelength range 0.69–1.95 Å, giving 2,193 unique reflections from the total of 3,817 obtained from the AFSCALE output. The results of data reduction are summarized below.

	p21c · GTP	p21c · GTP/GDP
R_{merge}(1, 2, 3) (see ref. 1)	7.8/7.6/7.2	7.4/7.2/5.6
Reflections (total)	3,817	4,338
Reflections (unique)	2,193	2,322
d (% of possible)	2.8 Å (50%)	2.8 Å (50%)

Milburn *et al.* have also compared the structures of the p21 · GDP and p21 · GPPCP complexes[19]. In agreement with our results, the main differences seen by this group are in the effector loop and in loop L4. Although some of these changes seem to be similar to those found in the present study, there are many differences in detail. This applies to both of the loops, and to the magnesium coordination. A thorough comparison of the results obtained will depend on the availability of coordinates for all of the relevant structures involved.

(5) p21 · caged GTP—'incorrect' binding in the crystal due to steric hindrance and crystal packing forces. The binding of caged GTP to p21 in the crystal differs from that of GTP, GPPNP or GDP (Fig. 3*a*). None of the main interactions seen with the other nucleotides is seen with caged GTP. First, the base is too far from Asp 119 for the strong interactions with N1 and N2 present in the other structures to occur. Phe 28 is also incorrectly orientated for an interaction with the aromatic ring system. These two interactions are thought to be of prime importance for positioning (Phe 28, together with the aliphatic side chain of Lys 116) and discrimination (Asp 119) of the base from the other nucleotides. In the triphosphate part of the molecule, the ribose is at roughly the same position as in the other complexes, but the α-, β- and γ-phosphates are far removed. Because of this, the important interactions of the latter two groups with the protein and with magnesium cannot occur. There are interactions of the 3′-hydroxyl group with the carbonyl group of Val 29, and of the oxygens of the γ-phosphate group with the amide hydrogens of Tyr 32 and Asp 33. None of these interactions is seen in the complexes between p21 and GPPNP, GTP or GDP. There seems to be an aromatic interaction of the cage group with Tyr 32 and of the nitro group with the side-chain carbamoyl group of Asn 86 of the neighbouring p21 molecule. It is not known whether magnesium is bound in this complex, but it seems likely that electron density associated with Ser 17 arises from the magnesium ion. There are some differences in the binding of caged GTP to p21(G12V) in the crystal. The nucleotide is twisted even further from its position in the other complexes. In contrast to the p21c · caged GTP complex, there is an interaction between the amino group of Lys 16 and the β-phosphate group.

The unusual mode of binding of caged GTP in the crystals prompted a more detailed investigation of the kinetics of interaction of caged GTP with p21 in solution. We have previously reported that it binds about 100-fold more weakly than GDP (ref. 9). A more detailed study, using a stopped-flow kinetic method[20], has led to values for the association rate constant ($2.5 \times 10^6\ M^{-1}\ s^{-1}$) and the dissociation rate constant ($2.5 \times 10^{-4}\ s^{-1}$; both values at 25 °C). The corresponding values for GDP are $8.5 \times 10^5\ M^{-1}\ s^{-1}$ and $1.2 \times 10^{-5}\ s^{-1}$, respectively[20]. Thus, from the quotient of the rate constants, caged GTP binds only a factor of ten more weakly than GDP (that is, $10^{10}\ M^{-1}$

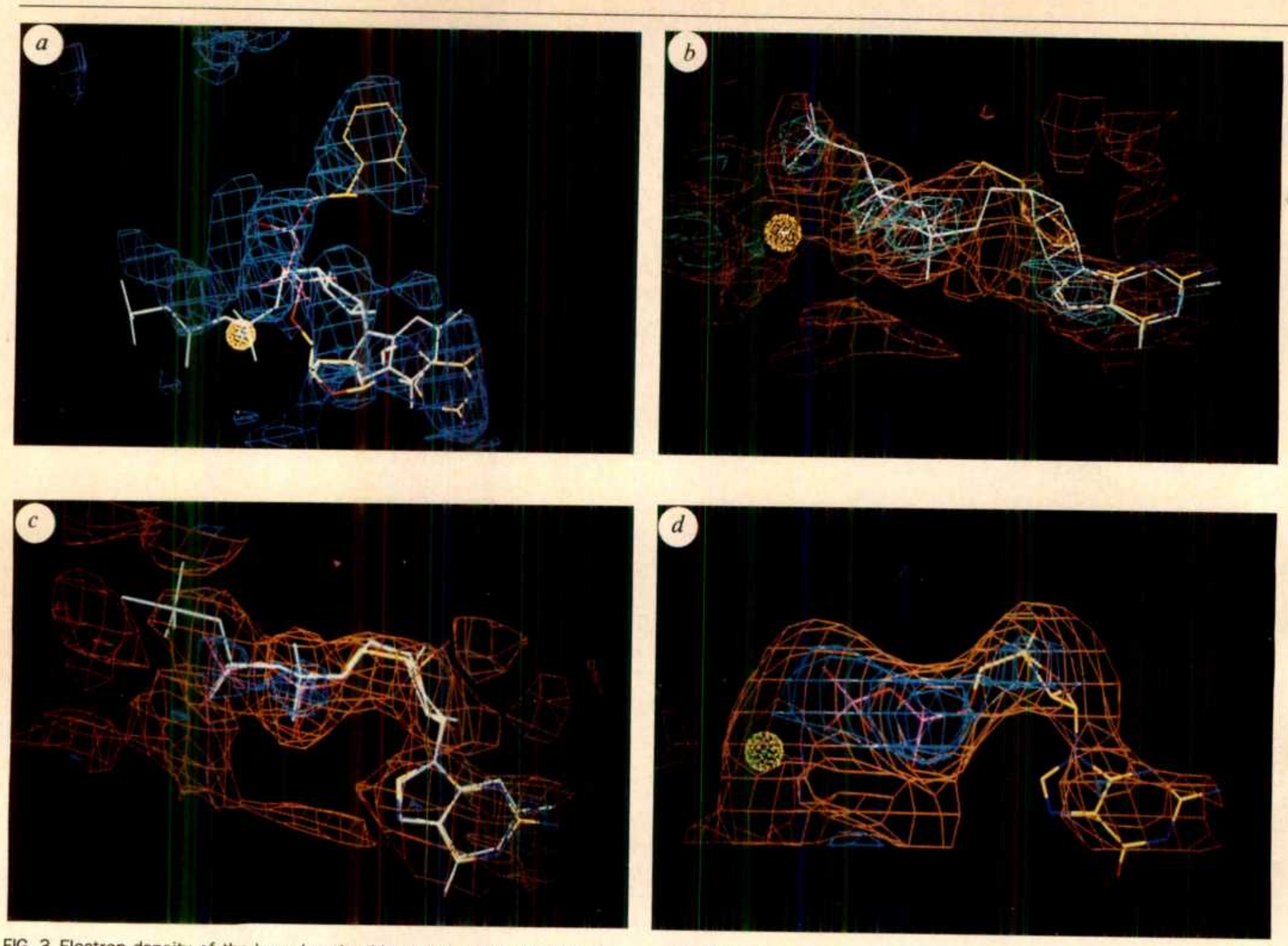
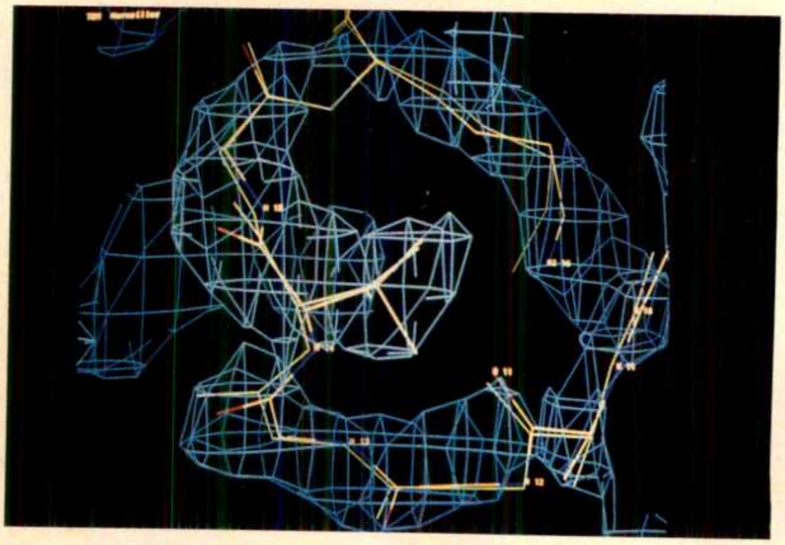


FIG. 3 Electron density of the bound nucleotide at the active site of p21c. *a*, Caged GTP. *b*, GTP, Laue data obtained 4 min after photolysis. *c*, GTP/GDP, Laue data obtained 14 min after photolysis. *d*, GDP. Electron density levels (blue, yellow respectively) are given as percentage of the maximum electron density: *a*, 45; *b*, 50, 22; *c*, 45, 10; *d*, 50, 22. Magnesium is shown as a sphere. In *a*, the structure of GTP (white) from *b* is superimposed on the caged GTP structure to show the different mode of binding of the latter. In *b*, the structure of GPPNP (ref. 13) is shown in white superimposed on the GTP structure. In *c*, the GTP structure (white) from *b* is superimposed on the GDP structure. It can be seen that there is much less density at the position of the γ-phosphate. The loss of density is greater than the ~30% expected from the rate constant for GTP cleavage. This effect could be caused by heating of the crystal in the white X-ray beam, which occurs at a significant rate. No other density that could be assigned to that missing from the terminal phosphate group was found. Refinement was performed with XPLOR (ref. 32) using the coordinates of the p21·GPPNP structure as a starting model[13]. About 100 water molecules were fitted in the p21c·GTP structure. Refinement parameters are given below.

	p21c				p21(G12V)		
	Caged GTP	GTP	GTP/ GDP	GDP	Caged GTP	GTP	GDP
R_{ini}	46.2	30	40	46.8	53.7	33.6	41.8
N_{cyc}	4	6	1	3	1	2	2
R_{fin}	19.9	14.0	14	18.5	25.3	21.4	17.9

N_{cyc} represents the number of XPLOR runs, each consisting of simulated annealing and energy/X-ray refinement with manual rebuilding using FRODO (ref. 33) between runs. $2F_0 - F_c$ electron density maps were calculated. R_{fin} is given in per cent (water molecules not included).

FIG. 4 Electron density (at 16% of the maximum value) in the p21c·GTP structure at the glycine-rich phosphate-binding loop. The good agreement between the structures of p21 complexed to GTP and GPPNP (in light green; ref. 13) is apparent.



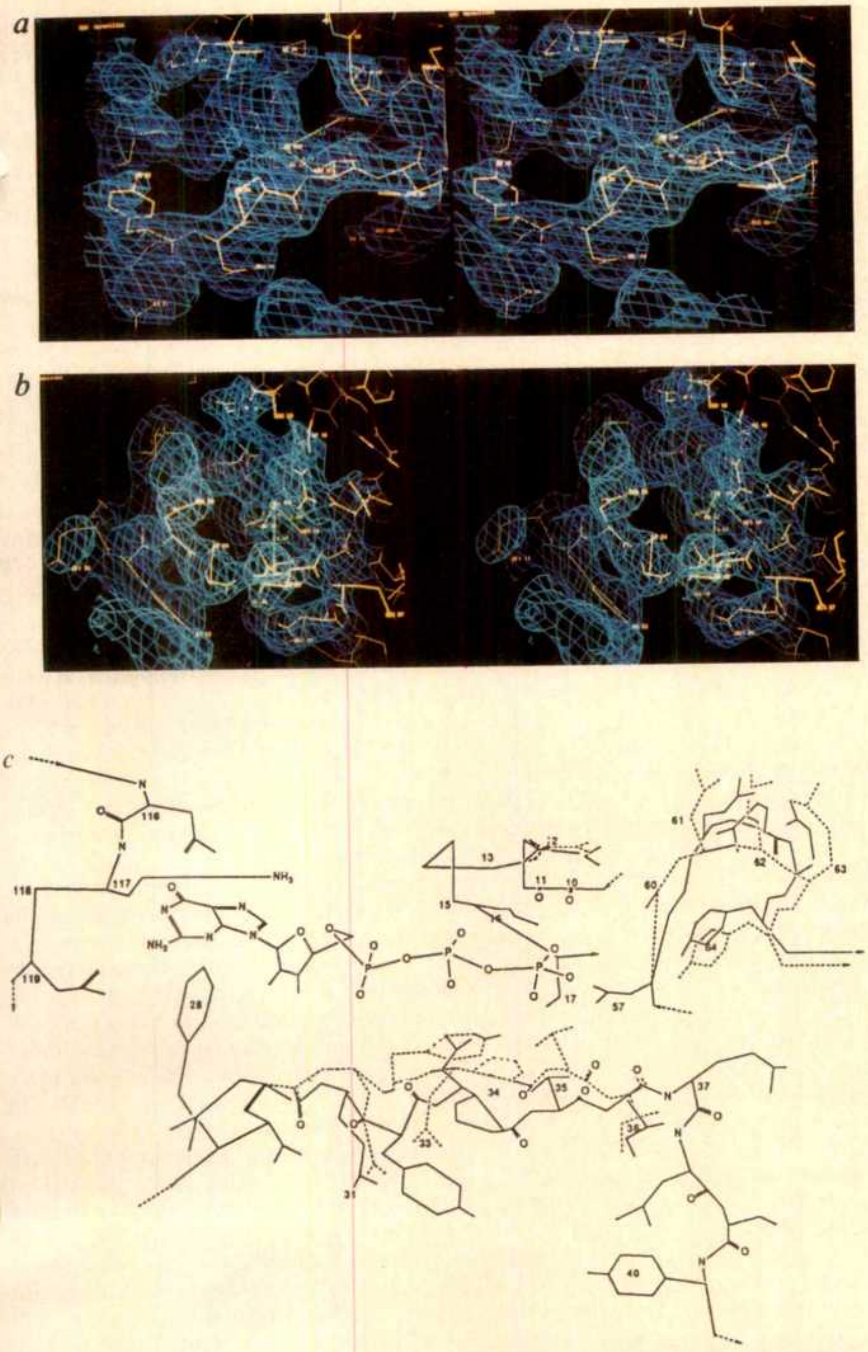


FIG. 5 Stereo view of the electron density (shown at 16% of the maximum value) of the effector region of p21(G12V) complexed to GTP (*a*) and GDP (*b*). There is a large movement of the residue 32–36 region between the two complexes. This is clarified in the schematic diagram *c*. The structure shown is the p21 · GTP complex, with the main changes in the p21 · GDP complex shown by the dashed lines.

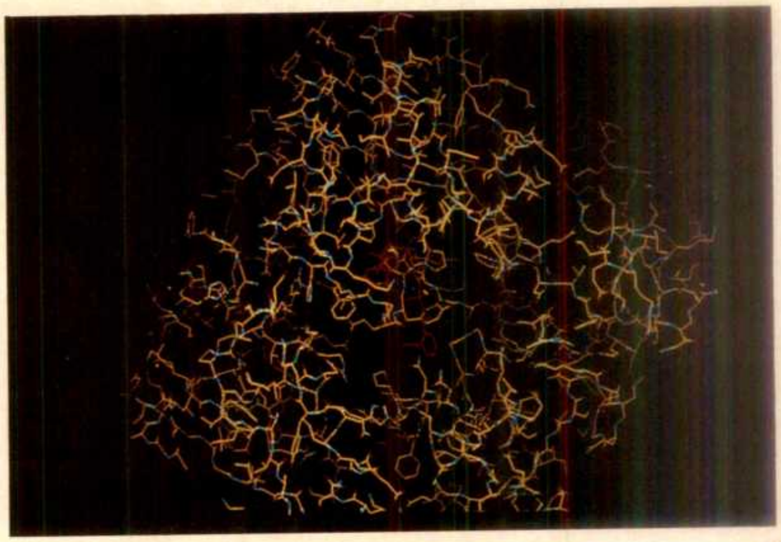


FIG. 6 Binding of caged GTP. The protecting 2-nitrophenylethyl group points towards a solvent channel (~15 × 20 Å) parallel to the *a*-axis of the crystal lattice.

compared with 10^{11} M^{-1}). This is difficult to reconcile with the observed mode of binding of caged GTP in the crystal. In addition, as with GDP and GTP, there is a marked effect of Mg^{2+} on the dissociation kinetics of caged GTP from p21, which does not accord with the apparent lack of interaction of Mg^{2+} with caged GTP at the active site of p21. Further evidence for the 'correct' binding of caged GTP in solution comes from two-dimensional NMR measurements on the p21 · caged GTP complex (data not shown). In particular, the distance between H1′ of the ribose ring and Phe 28 is found to be ~3.5 Å in solution (similar to GDP and GPPNP; ref. 21 and unpublished results), compared with 6 Å in the crystal structure. It is apparent from the structures seen after removal of the protecting group, that this altered mode of binding in the crystal does not cause any obvious problems for these experiments. In future experiments the unusual binding mode may even be advantageous. It could allow characterization of substrate-induced structural changes on correct binding of photogenerated GTP. We expect these to occur at a rate constant of $\sim 30\ s^{-1}$ at 25 °C ($\sim 5\ s^{-1}$ at 4 °C) from kinetic experiments in solution[20].

Modelling of caged GTP in the active site of p21 (keeping the GTP part of the analogue in the same position as in the p21 · GPPNP or p21 · GTP complexes) provides an explanation for these observations. It is seen that this would cause the protecting group to collide with the functional groups of Gln 61 and Tyr 32 of the neighbouring p21 molecule in the crystal structure. This seems to force the caged GTP molecule out of its preferred binding position, and indicates that the crystal packing energy must be greater than the binding energy of the analogue in its proper GTP-like position. In the new binding position, there is no steric hindrance of the protecting group (Fig. 6).

(6) Comparison of p21c and p21(G12V). The structures of p21c and p21(G12V) are very similar over most of the molecule in both the GTP and GDP forms. In particular, a similar conformation of the effector loop is seen in the respective GDP forms, and the same structural change occurs on hydrolysis to GTP. This similarity accords with the observations that the GTP form of p21(G12V) can bind to GAP with an affinity of the same order of magnitude as that of p21c, and that its affinity for GAP in the GDP form is much lower, as for p21c (refs 7, 8). There are, however, differences in the structures in the region of the phosphate-binding loop (although we do not observe the large widening of the loop as reported in ref. 22) and in the loop L4, the latter being pushed further away from the γ-phosphate group. This is particularly noticeable in the larger distance between the phosphate oxygens, the amide proton of Gly 60 and the carbonyl oxygen of Thr 58 on the one hand, and the side-chain of Gln-61 on the other hand. It is likely that these changes are associated with the reduced rate of the GTPase, particularly in the presence of GAP, in this mutant compared with p21c. The detailed analysis of transforming mutant protein structures at higher resolution than that available in the present study provides more information on these effects (U. Krengel *et al.*, manuscript submitted).

Discussion

Although it is not possible to draw detailed conclusions about the mechanism of GTP hydrolysis at the active site of p21, general features can be deduced from the available evidence. First, as is apparent both from this work and from the results on the structure of the p21 · GPPNP complex, there are many interactions between the β- and γ-phosphate groups, the protein and Mg^{2+}. These interactions both increase the electrophilicity of the γ-phosphate group (and therefore its susceptibility to attack by a water molecule) and improve the leaving group properties of the β-phosphate. These features are presumably shared by many other enzymes that catalyse phosphate transfer, as many of these have the conserved phosphate-binding loop (residues 10–18) characteristic of such enzymes[23,24]. Lys 16 seems to play a direct part in the catalytic mechanism. It is an invariant feature of the phosphate-binding loop and is always followed directly, or after an additional glycine, by a serine or threonine residue, in which p21 and presumably also in the other phosphate-transferring proteins, is complexed to Mg^{2+}. Preliminary modelling studies indicate that the interaction between the side-chain amino group of Lys 16 and the γ-phosphate group becomes even stronger in the putative pentacovalent intermediate, formed after the attack of water at phosphorous known to be direct from stereochemical studies[25]. The side chain of Lys 16 seems to be held in position by strong interactions with the main-chain carbonyl groups of residues 10 and 11. The structure envisaged would be very similar to the putative structure of transition-state adenylate kinase[35], ribonuclease A (ref. 26) and the complex between creatine kinase, ADP, a planar nitrate ion and creatine[27].

A water molecule has been identified in the p21 · GTP structure presented here and the high resolution p21 · GPPNP structure[34] that is likely to be the one that attacks the phosphate group. The similarity between these two structures and the explanation of the difference in the affinities of p21 for GTP and GPPNP justifies the derivation of biological and structural conclusions from the 121 · GPPNP structure.

Some indications of the mechanism of activation of the attacking water molecule can be derived from the available structures (as mentioned already) and are discussed in more detail elsewhere[34]. Further time-resolved studies using caged GPPNP, caged GTP (γ-S) and caged GTP should provide more information about this process.

Further studies are needed to clarify the structural change seen in the effector loop on GTP hydrolysis. Several technical improvements will help. First, changes in the nature of the photolytic protecting groups used could lead to structures at higher resolution for the various states characterized in this work. An obvious improvement is to use single diastereomers of caged GTP, which could lead to improvement of crystal quality, allowing time-resolved crystallography at higher spatial resolution. The use of a more powerful light source (laser) will improve the temporal resolution immediately after initiation of GTP hydrolysis. This could be complemented by use of a more sensitive detector system (probably imaging plates), that should lead to a reduction of at least a factor of ten in exposure time and increase (by a factor of ten or more) the number of data sets available from a single crystal. In this study, the highest number of data sets obtained before radiation damage became unacceptable was two. Another advantage is that the crystal heating by the white X-ray beam would also be reduced by a similar factor. A combination of these features, with increased X-ray intensity (available at several synchrotron sources) will extend investigations such as these into the sub-second and even sub-microsecond time range[28]. The work described here demonstrates that photolytic generation of substrates at the active site of a protein in the crystalline state is a viable approach to rapid initiation of an enzymate reaction, which is needed to take advantage of the potential of synchrotron radiation for time-resolved studies.

Coordinates of the p21 · GTP structure from the 1.35-Å electron density map will be deposited in the Brookhaven Data Bank. □

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1. Barbacid, M. *A. Rev. Biochem.* **56,** 779–827 (1987).
2. Gilman, A. G. *A. Rev. Biochem.* **56,** 615–649 (1987).
3. Allende, J. E. *FASEB J.* **2,** 2356–2367 (1988).
4. McCormick, F. *Cell* **56,** 5–8 (1989).
5. Adari, H., Lowy, D. R., Willumsen, B. M., Der, C. J. & McCormick, F. *Science* **240,** 518–521 (1988).
6. Cales, C., Hancock, J. F., Marshall, C. J. & Hall, A. *Nature* **332,** 548–551 (1988).
7. Marshall, M. S. *et al. EMBO J.* **8,** 1105–1110 (1989).
8. Vogel, U. S. *et al. Nature* **335,** 90–93 (1988).
9. Schlichting, I. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 7687–7690 (1989).
10. Moffat, K. *A. Rev. Biochem.* **18,** 309–332 (1989).
11. Hajdu, J. & Johnson, L. N. *Biochemistry* **29,** 1669–1678 (1990).
12. Scherer, A. *et al. J. molec. Biol.* **206,** 257–259 (1989).

13. Pai, E. F. *et al. Nature* **341,** 209–214 (1989).
14. Neal, S. E., Eccleston, J. F. & Webb, M. R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
15. Jurnak, F. *Science* **230,** 32–36 (1985).
16. Woolley, P. & Clark, B. F. C. *BioTechnology* **7,** 913–920 (1989).
17. De Vos, A. M. *et al. Science* **239,** 888–893 (1989).
18. Gibbs, J. B., Schaber, M. D., Allard, W. J., Sigal, I. S. & Scolnick, E. M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 5026–5030 (1988).
19. Milburn, M. V. *et al. Science* **247,** 939–945 (1990).
20. John, J., Sohmen, R., Feuerstein, J., Wittinghofer, A. & Goody, R. S. *Biochemistry* (in the press).
21. Schlichting, I. *et al. Biochemistry* **29,** 504–511 (1990).
22. Tong, L., Milburn, M., DeVos, A. M. & Kim, S. H. *Nature* **337,** 90–93 (1989).
23. Gay, N. J. & Walker, J. E. *Nature* **301,** 262–264 (1983).
24. Leberman, R. & Egner, U. *EMBO J.* **3,** 339–341 (1984).
25. Feuerstein, J., Goody, R. S. & Webb, M. R. *J. biol. Chem.* **264,** 6188–6190 (1989).
26. Deakyne, B. & Allen, J. *Am. chem. Soc.* **101,** 3951–3959 (1979).
27. Kenyon, G. L. & Reed, G. H. *Adv. Enzym.* **54,** 367–426 (1983).
28. Szebenyi, D. M. E. *et al. Trans Am. crystallogr. Ass.* **24,** 167–172 (1988).
29. Farber, G. K., Machin, P., Almo, S. C., Petsko, G. A. & Hajdu, J. *Proc. natn. Acad. Sci. U.S.A.* **85,** 112–115 (1988).
30. Hajdu, J. *et al. Nature* **329,** 178–181 (1987).
31. Helliwell, J. R. *et al. J. appl. Crystallogr.* **22,** 483–497 (1989).
32. Brünger, A. T., Kuriyan, K. & Karplus, M. *Science* **235,** 458–460 (1987).
33. Jones, T. A. *J. appl. Crystallogr.* **11,** 268–272 (1978).
34. Pai, E. F. *et al. EMBO J.* (in the press).
35. Reinstein, J., Schlichting, I. & Wittinghofer, A. *Biochemistry* (in the press).

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Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle

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Dystrophin, the protein encoded by the Duchenne muscular dystrophy (DMD) gene, exists in a large oligomeric complex. We show here that four glycoproteins are integral components of the dystrophin complex and that the concentration of one of these is greatly reduced in DMD patients. Thus, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein, and the reduction in this glycoprotein may be one of the first stages of the molecular pathogenesis of muscular dystrophy.

DUCHENNE muscular dystrophy is caused by a defective gene located on the X chromosome. Dystrophin, the high-molecular weight protein product of the DMD gene[1], is localized to the sarcolemmal membrane of normal skeletal muscle[2-5] but is absent from the skeletal muscle of people with DMD[1,2,6], *xmd* dogs[7] and *mdx* mice[1,5] (the last two being possible animal models for DMD). The amino-acid sequence of dystrophin suggests that it is a membrane cytoskeletal protein[8,9] involved in the anchoring of sarcolemmal proteins to the underlying cytoskeleton. But the exact function of dystrophin and its precise role in the resulting necrosis of dystrophic muscle fibres has not been determined. In studies of other genetic diseases involving proteins of the cytoskeleton[10,11], the absence of one component is sometimes accompanied by the loss of another cytoskeletal protein. Therefore, to understand the molecular pathogenesis of DMD, we sought to identify the proteins associated with or bound to dystrophin and to characterize the status of these proteins in muscle where dystrophin is absent.

Recently, we have shown that dystrophin can be isolated from detergent-solubilized skeletal muscle membranes using wheat-germ agglutinin (WGA)-Sepharose, because of its tight association with a WGA-binding glycoprotein[12]. This indicates that the localization of dystrophin to the cytoplasmic face of the sarcolemma[2-5] results from a tight association of dystrophin with an integral membrane glycoprotein. Here we report the purification of a large oligomeric complex (~18S) containing dystrophin using sucrose density-gradient centrifugation in the presence of digitonin. We have identified four glycoproteins of apparent relative molecular masses (M_r) 156,000 (156K), 50K, 43K and 35K as integral components of the dystrophin complex. The 156K and 50K glycoproteins are sarcolemmal glycoproteins, as shown by indirect immunofluorescence. Immunoaffinity beads raised against dystrophin and the 50K glycoprotein selectively adsorb the dystrophin–glycoprotein complex. Furthermore, there is a marked reduction of the 156K glycoprotein in muscle from *mdx* mice and DMD patients. These results imply that in dystrophic muscle, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein. This could be the first step in the molecular pathogenesis of muscular dystrophy.

Dystrophin–glycoprotein complex

This complex was isolated following digitonin-solubilization of rabbit skeletal muscle membranes using WGA–Sepharose and DEAE-cellulose[12] and further purified by sucrose density gradient centrifugation in the presence of 0.1% digitonin. It is evident from the Coomassie blue-stained gel of sequential gradient fractions (Fig. 1*a*) that the dystrophin–glycoprotein complex was separated from the voltage-sensitive sodium channel and the dihydropyridine receptor (Fig. 1). The size of the dystrophin complex was ~18S in comparison with β-galactosidase (15.9S), thyroglobulin (19.2S) and dihydropyridine receptor (20S) standards. Densitometer scanning of the peak dystrophin-containing fractions (10 and 11, Fig. 1*a*) revealed several proteins that co-purified with dystrophin: a broad, diffusely staining component with an apparent M_r of 156K, an 88K protein, a triplet of proteins centred at 59K, a 50K protein, a doublet at 43K and proteins of 35K and 25K.

To identify the glycoprotein constituents of the dystrophin–glycoprotein complex, sucrose gradient fractions 7–17 were electrophoretically separated, transferred to nitrocellulose and stained with peroxidase-conjugated WGA (Fig. 1*b*). Four WGA-binding proteins with apparent M_r of 156K, 50K, 43K and 35K were found to strictly co-purify with dystrophin. All four proteins were also positively stained with peroxidase-conjugated concanavalin A. In addition, the lower M_r component of the 43K protein doublet (Fig. 1*a*) was also positively stained with concanavalin A (not shown).

The dystrophin–glycoprotein complex was further characterized with antibodies raised against various components of the

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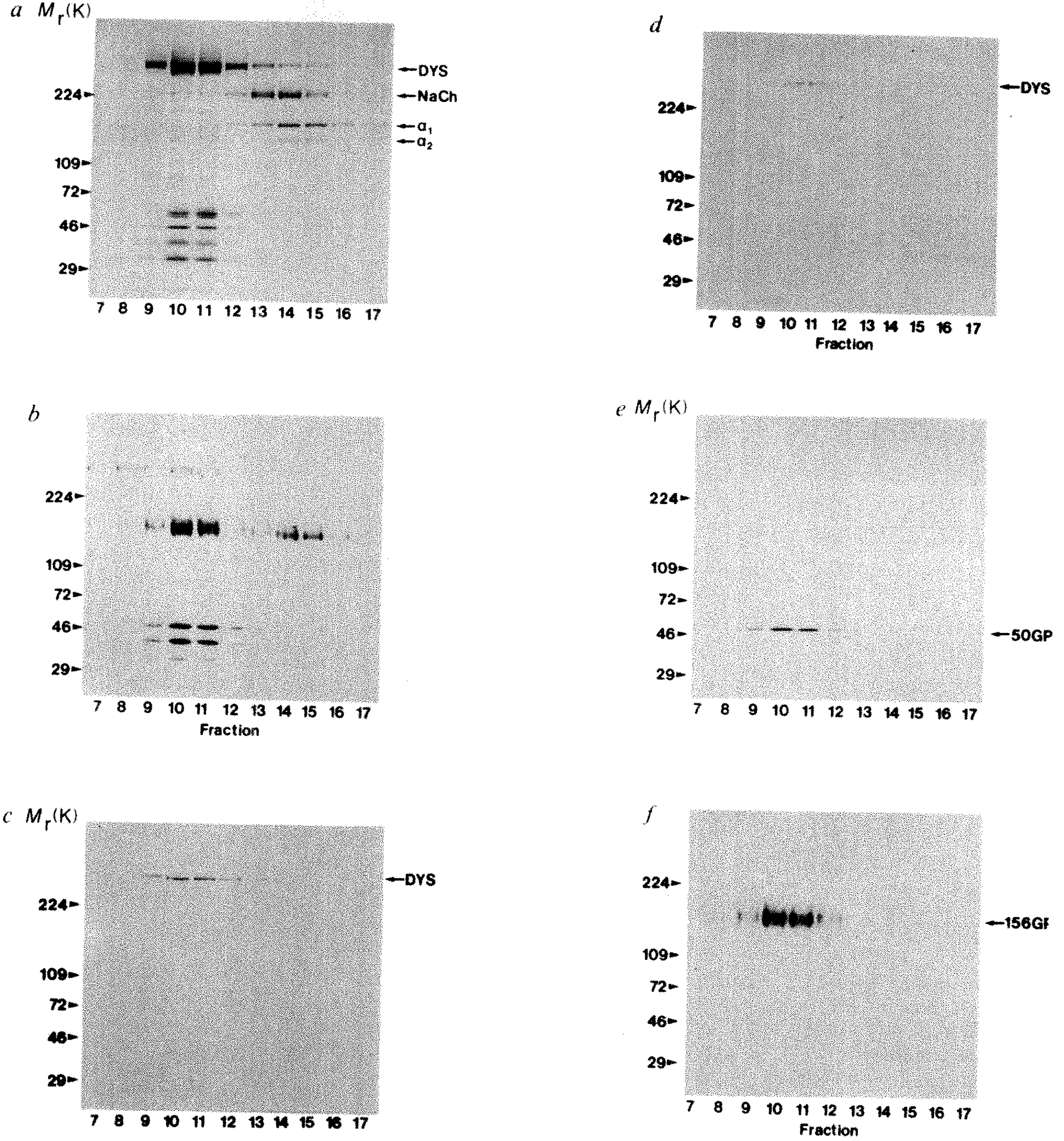

FIG. 1 Sedimentation of dystrophin complex through 5% to 20% linear sucrose gradients. *a*, Coomassie blue-stained gel of sucrose gradient fractions 7–17. *b*–*f*, Nitrocellulose transfers of sucrose gradient fractions 7–17 separated by SDS-PAGE and stained with: *b*, peroxidase-conjugated WGA (1 μg ml^{-1}); *c*, polyclonal antisera against the C-terminal decapeptide of dystrophin; *d*, monoclonal antibody (mAb) XIXC2 against dystrophin; *e*, mAb IVD3$_1$ against 50K glycoprotein (50 GP); or *e*, mAb VIA4$_1$ against the 156K glycoprotein (156 GP). Arrows indicate the positions of dystrophin (DYS), the voltage-sensitive sodium channel (NaCh), and the α_1 and α_2 subunits of the dihydropyridine (DHP) receptor. Arrowheads denote the positions of the molecular weight standards as indicated.

METHODS. Heavy microsomes were prepared from rabbit skeletal muscle[25] and washed twice with 0.6 M KCl in 50 mM Tris-HCl (pH 7.4), 0.165 M sucrose, 0.1 mM phenylmethylsulphonyl fluoride and 0.75 mM benzamidine to remove contractile proteins. KCl-washed membranes (1 g) were solubilized in 1.0% digitonin, 0.5 M NaCl, and protease inhibitors as previously described[12]. After removal of the ryanodine receptor by immunoaffinity chromatography[26], the digitonin-solubilized membranes were circulated overnight on a 40 ml WGA-Sepharose column, washed extensively, then eluted with three column volumes of 0.3 M *N*-acetylglucosamine. Eluted fractions containing dystrophin were applied to a 3-ml DEAE-cellulose column and sequentially eluted with the following NaCl concentrations in buffer A (0.1% digitonin, 50 mM Tris-HCl, pH 7.4, 0.75 mM benzamidine, 0.1 mM PMSF): 0, 25, 50, 75, 100, 110 and 175 mM. Sucrose gradients (12.5 ml linear 5–20% sucrose) containing 0.5 M NaCl and 0.01% NaN$_3$ in buffer A were prepared using a Beckman density gradient former. Dystrophin complex, which eluted in fraction 2 (3 ml) from the DEAE-column 175 mM NaCl wash, was concentrated to 0.5 ml in a Centricon-100 (Amicon), layered on a sucrose gradient and overlain with 0.5 ml of buffer A containing 175 mM NaCl and 0.01% NaN$_3$. Gradients were centrifuged at 4 °C in a Beckman VTi 65.1 vertical rotor for 90 min at 200,000*g*. Fractions (0.6 ml) were collected from the top of the gradients using an ISCO Model 640 density gradient fractionator. Gradient fractions were separated by SDS-PAGE[27] (3–12% gradient gel) and stained with Coomassie blue (300 μl concentrated with a Centricon-100) or transferred to nitocellulose (75 μl of fractions in *b*, 25 μl in *c* and *d*, and 50 μl in *e* and *f*) and stained with various antibodies. The blot shown in *e* was prepared from a gel run in the absence of reducing agent plus 10 mM *N*-ethylmaleimide. Gel lanes were scanned with a Hoefer GS 300 scanning densitometer and analysed using GS-360 data analysis software. Polyclonal antisera against a chemically synthesized decapeptide representing the C-terminal of dystrophin was raised in New Zealand white rabbits as described[28]. Hybridomas were obtained from female BALB/c mice which had been immunized with rabbit skeletal muscle membranes and boosted with WGA eluate[29].

complex. Antisera from a rabbit immunized with a chemically synthesized decapeptide representing the predicted C-terminal amino-acid sequence of human dystrophin, stained a single high-M_r protein (Fig. 1*c*). This protein co-migrated with the predominant isoform of dystrophin stained by sheep polyclonal anti-dystrophin antibodies[13] (not shown). The antisera showed immunofluorescence staining only on the cell periphery (Fig. 2*a*), which indicates a restricted localization of dystrophin to the sarcolemma of rabbit skeletal muscle.

A library of monoclonal antibodies against muscle proteins eluted from WGA-Sepharose was also screened for reactivity against components of the dystrophin-glycoprotein complex and by indirect immunofluorescence staining of rabbit skeletal muscle. Of six hybridomas which showed immunofluorescence staining only on the sarcolemma, monoclonal antibodies XIXC2 (Fig. 1*d*) and VIA4$_2$ (not shown) were found to stain dystrophin on immunoblots. Both dystrophin monoclonal antibodies are IgM subtypes, and recognized both native and denatured dystrophin. Monoclonal antibody XIXC2 also recognizes the minor lower-M_r isoform of dystrophin which co-purifies with the more abundant isoform (Fig. 1*d*).

Two of the other sarcolemma-specific monoclonal antibodies were specific for components of the dystrophin-glycoprotein complex (Fig. 1*e* and 1*f*). The 50K glycoprotein stained with monoclonal antibody IVD3$_1$ (Fig. 1*e*), and has been localized exclusively to the sarcolemmal membrane of rabbit skeletal muscle (Fig. 2*c*). Monoclonal antibody IVD3$_1$ recognized only the non-reduced form of the 50K glycoprotein and is not highly cross-reactive. Monoclonal antibody VIA4$_1$ stained the 156K glycoprotein (Fig. 1*f*) which co-purified with dystrophin. VIA4$_1$ recognized the denatured form of the 156K glycoprotein and is highly cross-reactive. It also exhibited weak, but specific immunofluorescent staining of the sarcolemmal membrane (Fig. 2*d*), consistent with its low affinity for the native 156K glycoprotein. In agreement with the immunofluorescence results, a rabbit membrane preparation greatly enriched in sarcolemmal proteins also showed a substantial enrichment in dystrophin, the 156K and 50K glycoproteins (not shown). Immunofluorescence staining for dystrophin, 50K glycoprotein or the 156K glycoprotein was equally distributed in fast and slow muscle fibres (not shown).

The association of the dystrophin-glycoprotein complex was also assessed by immunoaffinity adsorption. Immunoaffinity beads were prepared with the monoclonal antibodies XIXC2 (anti-dystrophin) and IVD3$_1$ (anti-50K glycoprotein) and incubated with the partially purified dystrophin-glycoprotein complex. After pelleting the immunoaffinity beads, the supernatants were removed and the beads were washed extensively. The supernatants and washes were pooled (voids), concentrated, and analysed by SDS-polyacrylamide gel electrophoresis and immunoblotting. The voids from the XIXC2 (anti-dystrophin) and the IVD3$_1$ (anti-50K glycoprotein) immunoaffinity beads contained no dystrophin, 59K triplet, 50K, 43K doublet or 35K proteins as detected by Coomassie blue staining (Fig. 3*a*). Both the XIXC2 (anti-dystrophin) and IVD3$_1$ (anti-50K glycoprotein) immunoaffinity beads quantitatively removed dystrophin from the starting material (Fig. 3*c*). Analysis of the voids for the 156K (Fig. 3*d*) and 50K (Fig. 3*e*) glycoproteins revealed that both the XIXC2 and IVD3$_1$ immunoaffinity beads selectively adsorbed virtually all of each of these glycoproteins from the voids, whereas the voltage-sensitive sodium channel (Fig. 3*b*) and the α_1 and α_2 subunits of the dihydropyridine receptor (not shown) remained in the voids. As detected by peroxidase-conjugated WGA (not shown), the 43K and 35K glycoproteins were also adsorbed from the voids. Immunoblots of immunoaffinity beads separated on gels indicated that dystrophin, the 156K and 50K glycoproteins were retained by the beads and not selectively proteolysed (not shown). Initial experiments with monoclonal antibody VIA4$_1$ (anti-156K glycoprotein) have indicated that it has too low an affinity for the native 156K glycoprotein to be successful in this type of experiment.

Analysis of dystrophic muscle

To investigate whether either of the dystrophin-linked 156K or 50K glycoproteins is affected by the absence of dystrophin, immunoblots of skeletal muscle membranes were prepared from control and *mdx* mice and stained with the various antibodies

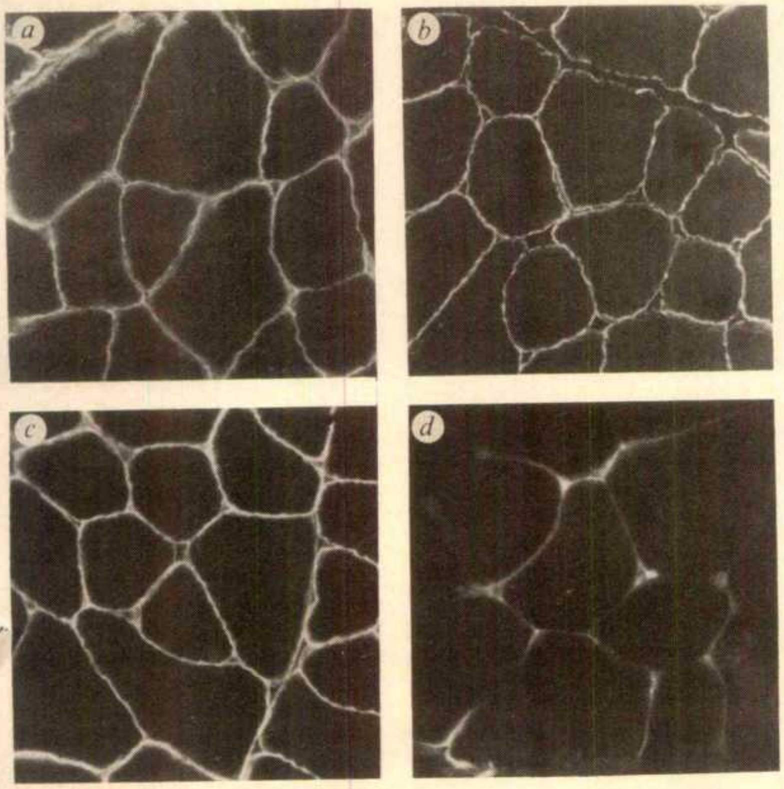

FIG. 2 Immunolocalization of components of the dystrophin complex. Transverse cryostat sections of rabbit skeletal muscle were labelled by indirect immunofluorescence with polyclonal antisera against the C-terminal decapeptide of dystrophin (*a*), mAb XIXC2 against dystrophin (DYS) (*b*), mAb IVD3$_1$ against the 50K glycoprotein (50 GP) (*c*) and monocolonal antibody VIA4$_1$ against the 156K glycoprotein (156 GP) (*d*) (magnification, 250×). Staining of the cryostat sections was not observed with nonimmune serum, nor was there any nonspecific binding to the tissue by fluorescein-labelled secondary antibody.

METHODS. The indirect immunofluorescence labelling of fixed 8-μm transverse cryostat sections from rabbit gastrocnemius was carried out as described[29]. Sections were pre-incubated for 20 min with 5% normal goat antiserum in PBS buffer, followed by a 2 h incubation at 37 °C with the primary antibody (hybridoma supernatants or 1:1,000 diluted antiserum). After washing in PBS, the sections were further incubated for 30 min at 37 °C in PBS with a 1:50 dilution of FITC-labelled goat F(ab')$_2$ anti-mouse IgG or anti-rabbit IgG and subsequently examined in a Leitz fluorescence microscope.

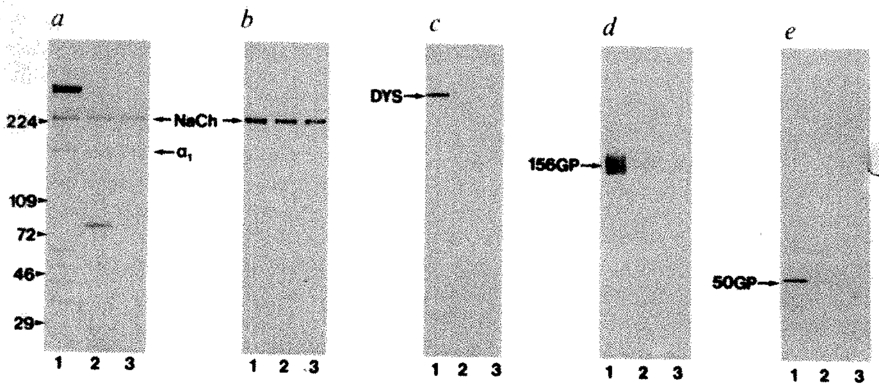

FIG. 3 Immunoadsorption of the dystrophin-glycoprotein complex. Fraction 2 (125 μl in *a*, 25 μl in *b*–*e*) eluted from the 175 mM NaCl wash of the DEAE-cellulose column described in Fig. 1 before treatment (lane 1), the XIXC2 affinity column void (125 μl in *a*, 25 μl in *b*–*e*) (lane 2) or the IVD3$_1$ affinity column void (25 μl) (lane 3), stained with Coomassie blue (*a*), mAb G/C6 against the sodium channel (NaCh) (*b*), polyclonal antisera to the C-terminal decapeptide of dystrophin (DYS) (*c*), mAb VIA4$_1$ (156 GP) (*d*), or mAb IVD3$_1$ (50 GP) (*e*). Molecular weight standards (arrowheads) were the same as those used in Fig. 1.

METHODS. Immunoaffinity beads[30] were equilibrated with buffer A containing 0.5 M NaCl and then incubated (12 h) with 0.75 ml of fraction 2 from the 175 mM NaCl wash of the DEAE-cellulose column (Fig. 1). After pelleting, the supernatants were decanted (voids) and the affinity beads were washed with 5 aliquots (0.7 ml) of buffer A containing 0.5 M NaCl. The void from each affinity column and the five washes were pooled and concentrated to 375 μl in a Centricon-100. In addition, 0.75 ml of fraction 2 was diluted to 4.2 ml, concentrated to 375 μl and used as control. Column voids were analysed by SDS-PAGE and immunoblotting as described in Fig. 1. Monoclonal antibody G/C6 against the skeletal muscle sodium channel[31] was the gift of Dr Robert Barchi (University of Pennsylvania).

(Fig. 4). Staining with polyclonal antisera against the C-terminal decapeptide of dystrophin revealed that dystrophin was completely absent from *mdx* mouse membranes (Fig. 4*a*). In addition, comparison of normal and *mdx* mouse with immunostaining by monoclonal antibody VIA4$_1$ against the 156K glycoprotein revealed that the 156K glycoprotein was absent or greatly reduced in *mdx* mouse membranes (Fig. 4*b*). Staining of identical transfers with sheep polyclonal antisera against either the ryanodine receptor (Fig. 4*c*) or the dihydropyridine receptor (Fig. 4*d*) did not differ between control and *mdx* mouse muscle membranes. Monoclonal antibody IVD3$_1$ against the 50K glycoprotein did not cross-react with normal mouse membranes and thus could not be evaluated. The absence of the 156K glycoprotein was also confirmed using SDS muscle extracts (not shown) instead of isolated membranes from control and *mdx* mice. Estimation of the amount of 156K glycoprotein remaining in the *mdx* muscle membranes using ^{125}I-labelled secondary antibodies and total membrane preparations from four control and four *mdx* mice revealed an average reduction of 85% in *mdx* muscle.

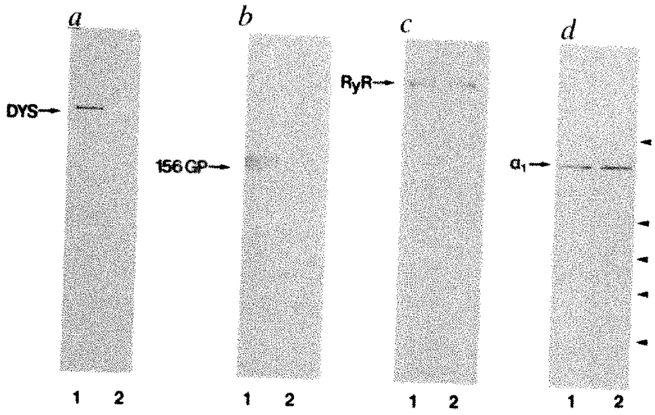

FIG. 4 Immunoblot analysis of control and *mdx* mouse muscle membranes. Immunoblots stained with polyclonal antisera against the C-terminal decapeptide of dystrophin (DYS) (*a*), mAb VIA4$_1$ against the 156K glycoprotein (156 GP) (*b*), sheep polyclonal anti-ryanodine receptor antibody (RyR) (*c*) and sheep polyclonal anti-DHP receptor antibody (α_1) (*d*) are shown. Lanes (1) and (2) for each panel consist of equal amounts of muscle membrane protein from control and *mdx* mice, respectively (300 μg per lane in (*a*) and (*b*), 150 μg per lane in (*c*) and (*d*)). Molecular weight standards (arrowheads) were the same as Fig. 1.

METHODS. Membranes from control and *mdx* mice (gifts of Dr Richard Strohmen and Dr Richard Entrikin of University of California, Berkeley and Davis, respectively) were prepared in 10% sucrose, 76.8 nM aprotinin, 0.83 mM benzamidine, 1 mM iodoacetamide, 1.1 μM leupeptin, 0.7 μM pepstatin A, 0.23 mM PMSF, 20 mM Tris-maleate, pH 7.0, by centrifuging muscle homogenates for 15 min at 14,000*g* and subsequently pelleting the supernatant for 30 min at 125,000*g*, followed by KCl washing as described in Fig. 1. Control and *mdx* mouse muscle membranes were analysed by SDS-PAGE and immunoblotting as described in Fig. 1. The amount of 156K glycoprotein in each preparation was estimated densitometrically from autoradiographs of identical blots incubated with ^{125}I-labelled sheep anti-mouse secondary antibody[32].

Total muscle extracts were also prepared from biopsy samples of normal controls and DMD patients (obtained from the Department of Neuropathology, University of Iowa). The dystrophic samples showed no staining with antibodies against dystrophin by indirect immunofluorescence microscopy (not shown) and immunoblotting (Fig. 5*a*). In contrast to the normal muscle extract, the three DMD samples showed greatly reduced staining for the 156K glycoprotein (Fig. 5*b*). In contrast, identical immunoblots stained with monoclonal antibodies against the Ca^{2+}-dependent ATPase (Fig. 5*c*) revealed no difference in the staining intensity between normal and dystrophic muscle samples. Again, the amount of 156K glycoprotein was reduced by about 90% in DMD samples.

Discussion

We have presented evidence for the existence of a large oligomeric complex (~18S) containing dystrophin, a 59K triplet

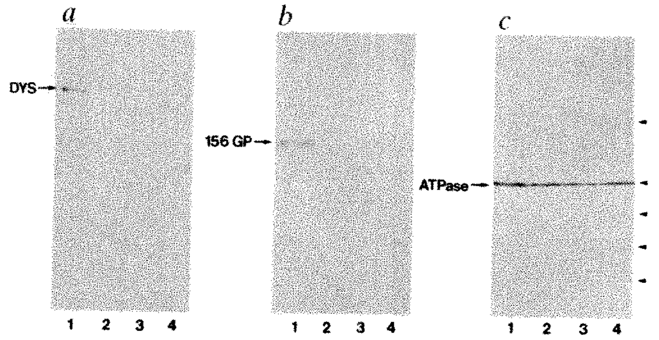

FIG. 5 Immunoblot analysis of normal and dystrophic human muscle biopsies. Immunoblots stained with mAb VIA4$_2$ against dystrophin (DYS) (*a*), mAb VIA4$_1$ against the 156K glycoprotein (156 GP) (*b*), or mAb IID8 against the (Ca^{2+}+Mg^{2+})-ATPase (ATPase) (*c*) are shown. Lane (1) consists of normal human muscle extract and lanes (2–4) are dystrophic muscle extracts from three DMD patients. Molecular weight standards (arrowheads) were the same as Fig. 1.

METHODS. Frozen muscle biopsy samples (50 mg) were crushed in liquid nitrogen using a mortar and pestle and then prepared for electrophoresis as described[6]. The pulverized muscle samples were transferred to 10 volumes of SDS-PAGE sample buffer (10% SDS, 2 M sucrose, 4% 2-mercaptoethanol, 0.002% bromophenol blue, 260 mM Tris-HCl, pH 6.8), vortexed, and precipitated material allowed to settle. Aliquots (50 μl) of the SDS-extracted muscle samples were analysed by SDS-PAGE and immunoblotting as described in Fig. 1 and the amount of 156K glycoprotein was estimated as described in Fig. 4.

and four sarcolemmal glycoproteins (156K, 50K, 43K and 36K). At least one of the proteins in the complex is an integral membrane protein, as 1.0% digitonin was necessary to solubilize the dystrophin–glycoprotein complex. The immunoaffinity experiments demonstrate that the complex is tightly associated. To date, a large number of antibodies specific for extracellular matrix proteins, cytoskeletal proteins, plasma membrane pump, channel and receptor proteins have been screened for reactivity against the dystrophin–glycoprotein complex. None of these antibodies to known proteins has demonstrated cross-reactivity to any component of the complex. The elucidation of primary sequences by recombinant DNA techniques should provide clues to the function of the dystrophin-associated glycoproteins.

The surface of DMD myofibres have been reported to show altered[14] or decreased[15] lectin binding, and a 370K glycoprotein is apparently missing from DMD muscle[16]. However, to our knowledge this is the first demonstration of the marked deficiency of a glycoprotein that is closely linked to dystrophin. The substantial reduction of the 156K glycoprotein from muscles of *mdx* mice and DMD patients is analogous to findings in erythrocytes of individuals afflicted with hereditary elliptocytosis[17], in which the absence of the cytoskeletal protein band 4.1[11,18,19] is accompanied by greatly diminished steady-state levels of glycophorin C[10,11]. In both diseases, the disruption of the cytoskeleton seems to destabilize the plasma membrane and associated proteins. As antibody probes to other components of the dystrophin complex become available, it will be interesting to determine if any other proteins are also affected in *mdx* and DMD muscle.

How the absence of dystrophin leads to the clinical manifestation of DMD is an unanswered question. Clearly there could be many steps in the disease process, yet we may have identified the first, which is the loss of a dystrophin-associated glycoprotein due to the absence of dystrophin. For example, muscle from *mdx* mice shows elevated intracellular ionized Ca^{2+} levels and corresponding higher net degradation of muscle proteins[20]. Loss of a dystrophin-anchored protein with a role in the regulation of intracellular calcium could result in elevated intracellular calcium levels and lead to the reported activation of calcium-dependent protease activities[21]. Such a mechanism could explain the abnormal muscle protein degradation and fibre necrosis of dystrophic muscle.

The absence of dystrophin-associated proteins in dystrophic muscle may complicate the therapeutic efficacy of myoblast transfer[22], as the reintroduction of dystrophin synthesis might not lead to recovery of associated protein levels and thus could necessitate treatment at only one particular developmental stage. Finally, a deficiency or defect in a dystrophin-associated glycoprotein could perhaps explain the DMD-like symptoms observed in suspected autosomal-recessive patients[23,24] that express apparently normal dystrophin. □

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1. Hoffman, E. P., Brown, R. H. & Kunkel, L. M. *Cell* **51**, 919–928 (1987).
2. Zubrzycka-Gaarn, E. E. *et al. Nature* **333**, 466–469 (1988).
3. Arahata, K. *et al. Nature* **333**, 861–866 (1988).
4. Bonilla, E. *et al. Cell* **54**, 447–452 (1988).
5. Watkins, S. C., Hoffman, E. P., Slayter, H. S. & Kunkel, L. M. *Nature* **333**, 863–866 (1988).
6. Hoffman, E. P. *et al. New Engl. J. Med.* **318**, 1363–1368 (1988).
7. Cooper, B. J. *et al. Nature* **334**, 154–156 (1988).
8. Koenig, M., Monaco, A. P. & Kunkel, L. M. *Cell* **53**, 219–228 (1988).
9. Davison, M. D. & Critchley, D. R. *Cell* **52**, 159–160 (1988).
10. Alloisio, N. *et al. Biochim. biophys. Acta* **816**, 57–62 (1985).
11. Mueller, T. J. & Morrison, M. *Erythrocyte Membrane 2: Recent Clinical and Experimental Advances* 95–112 (Liss, New York, 1981).
12. Campbell, K. P. & Kahl, S. D. *Nature* **338**, 259–262 (1989)
13. Knudson, C. M., Hoffman, E. P., Kahl, S. D., Kunkel, L. M. & Campbell, K. P. *J. biol. Chem.* **263**, 8480–8484 (1988).
14. Bonilla, E., Schotland, D. L. & Wakayama, Y. *Ann. Neurol.* **4**, 117–123 (1978).
15. Capaldi, M. J., Dunn, M. J., Sewry, C. A. & Dubowitz, V. *J. neurol. Sci.* **63**, 129–142 (1984).
16. Capaldi, M. J., Dunn, M. J., Sewry, C. A. & Dubowitz, V. *J. neurol. Sci.* **68**, 225–231 (1985).
17. Palek, J. *Clin. Haematol.* **14**, 45–87 (1985).
18. Alloisio, N., Dorleac, E., Girot, R. & Delaunay, J. *hum. Genet.* **59**, 68–71 (1981).
19. Tchernia, G., Mohandas, N. & Shohet, S. B. *J. clin. Invest.* **68**, 454–460 (1981).
20. Turner, P. R., Westwood, T., Regen, C. M. & Steinhardt, R. A. *Nature* **335**, 735–738 (1988).
21. Nagy, B. & Samaha, F. J. *Ann. Neurol.* **20**, 50–56 (1986).
22. Partridge, T. A., Morgan, J. E., Coulton, G. R., Hoffman, E. P. & Kunkel, L. M. *Nature* **337**, 176–179 (1989).
23. Francke, U., Darras, B. T., Hersh, J. H., Berg, B. O. & Miller, R. G. *Am. J. hum. Genet.* **45**, 63–72 (1989).
24. Zatz, M., Passos-Bueno, M. R. & Rapaport, D. *Am. J. med. Genet.* **32**, 407–410 (1989).
25. Mitchell, R. D., Palade, P. & Fleischer, S. *J. Cell Biol.* **95**, 1008–1016 (1983).
26. Imagawa, T., Smith, J. S., Coronado, R. & Campbell, K. P. *J. biol. Chem.* **262**, 16636–16643 (1987).
27. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
28. Strynadka, N. C. J., Redmond, M. J., Parker, J. M. R., Scraba, D. G. & Hodges, R. S. *J. Virol.* **62**, 3474–3483 (1988).
29. Jorgensen, A. O. *et al. Cell Motil. Cytoskel.* **9**, 164–174 (1988).
30. Campbell, K. P. *et al. J. biol. Chem.* **262**, 6460–6463 (1987).
31. Casadei, J. M. & Barchi, R. L. *J. Neurochem.* **48**, 773–778 (1987).
32. Sharp, A. H. & Campbell, K. P. *J. biol. Chem.* **264**, 2816–2825 (1989).

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Flat-spectrum radio sources: cosmic conspiracy or relativistic neutrons?

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RADIO-LOUD active galactic nuclei (AGNs) tend to show extended emission in the form of jets and an unresolved central core, from which the jets presumably originate. The intensity spectrum of the core varies smoothly from $10^{8.5}$ to 10^{16} Hz in frequency[1], and is flat (constant with frequency) between 10^9 and 10^{12} Hz, implying that a single emission mechanism, probably synchrotron radiation, is responsible. Because synchrotron emission below $\sim 10^{13}$ Hz would be self-absorbed for a uniform source under conditions typical of AGNs, inhomogeneous models have been used to account for these spectra. These models, which require the magnetic field strength and the electron density and energy to vary according to strict power laws, reproduce the flat spectra by a number of self-absorbed components[2–4], but they do not explain the origin of radiating electrons at $\sim 10^{18}$ cm from the AGN core, in view of the very short lifetime of the electrons in an AGN environment. Here we propose that energy is transported from the central source by relativistic neutrons, which travel freely over a large volume and decay into relativistic protons. The protons produce secondary electrons which generate the observed radiation. The photon spectra thus produced are largely model independent and flat.

Relativistic neutrons can result from the collisions of high-energy protons with the ambient gas[5], or high-energy protons with the ambient photon field if their energies are sufficiently large[6]. Actually, in high-energy pp collisions the leading particle carries most of the energy[7]. In about half of these interactions the leading proton is converted into a neutron, so the neutrons can carry away ~25–50% of the total luminosity available (here we assume that this fraction is 25%). But unlike protons, the relativistic neutrons are not tied to the ambient magnetic field; they readily escape from the system and traverse on the average a distance $r \approx 3 \times 10^{13}\ \gamma_n$ cm before they decay, γ_n being the neutron Lorentz factor. Therefore, if the neutron distribution extends to $\gamma_n \approx 10^5$, relativistic neutrons will reach as far as ~1 pc from the 'central engine'. The relativistic protons resulting from the neutron decay produce secondary relativistic electrons (and positrons) throughout this region *in situ*, thus successfully addressing the question of the origin of these particles at such large distances from the continuum source. In addition, the emission of radiation over $r \approx 1$ pc is intimately related, through the neutron transport, to the conditions in the central source, thus severely restricting the assumptions that can be made about the population of these relativistic radiating electrons.

We shall assume that at the 'central engine' there exists a steady-state relativistic proton distribution proportional to γ^{-s}, $s \approx 2$, that is, similar to that expected from a strong shock[8,9]. This steady state is considered to be the result of continuous injection of relativistic protons by the 'engine' and their removal by nuclear collisions[10]. As argued above, the proton distribution will give rise to a steady flux of neutrons $F_n(\gamma_n, r) \propto \gamma_n^{-s} r^{-2} \exp(-r/c\tau\gamma_n)$ cm^{-2} s^{-1}, where τ is the rest-frame lifetime of the neutron, ~10^3 s. The γ_n^{-s} factor reflects the spectrum of the primary protons responsible for the neutron production, the r^{-2} factor the geometric dilution, and the exponential factor their depletion through decay (there is, in general, another depletion due to their interactions with ambient gas but it is energy independent and has been omitted here for simplicity; it will be argued later, that this term must be insignificant to produce a radio-loud object).

The differential spectrum of protons resulting from the neutron decay Q_p, is equal to the number of neutrons decaying in a radius interval dr, or more precisely to the divergence of the neutron flux, $\nabla \cdot \mathbf{F}_n$. For spherical symmetry this is

$$Q_p(\gamma_p, r) = \frac{1}{r^2}\frac{d}{dr}[r^2 F_n(\gamma_n, r)]$$

$$= \left(\frac{r_0}{r}\right)^2 \frac{I_n}{c\tau}\gamma_p^{-(s+1)} \exp\left(\frac{-r}{c\tau\gamma_p}\right) \quad \text{cm}^{-3}\,\text{s}^{-1} \qquad (1)$$

where I_n is the normalization of the neutron flux at the radius r_0 (also bearing in mind that γ_p is almost identical to γ_n). At low energies, the proton injection spectrum has a kinematic cutoff at $\gamma_{p,b} \approx r/c\tau$ due to the decay of the lower-energy neutrons, which increases linearly with r. Above this cutoff the spectrum is steeper than the neutron spectrum by one power of γ, due to fewer decays of high-energy neutrons relative to low-energy neutrons in a shell of thickness dr. This extra steepening is important for obtaining a 'flat' radio spectrum.

The decay-produced relativistic protons couple to the ambient magnetic field and incorporate themselves into the local plasma which for simplicity is considered to be at rest (see, however, ref. 11). In a steady state they will deposit all their energy through nuclear collisions in the form of relativistic e^-e^+(~20%), π^0-decay γ-rays (~30%) and neutrinos (~50%). The Lorentz factor of the resulting electrons is $\gamma_e \approx 60\gamma_p$, and the energies of the γ-rays are $E_\gamma \approx 70\gamma_p$ MeV.

The resulting injection rate for secondary relativistic electrons, $Q_e(\gamma_e, r)$, is very similar to that of protons except that the kinematic cutoff is at a Lorentz factor $\gamma_{e,b} \approx 60\gamma_{p,b}$; its normalization is also different, determined by demanding that the secondary electrons, of $\gamma_e \approx 60\gamma_p$, carry ~20% of the proton luminosity. $Q_e(\gamma_e, r)$ is given approximately by

$$Q_e(\gamma_e, r) \approx 2.14 \times 10^4 \left(\frac{r_0}{r}\right)^2 \frac{I_n}{c\tau}\gamma_e^{-(s+1)}$$

$$\times \exp\left(\frac{-60r}{c\tau\gamma_e}\right) \quad \text{cm}^{-3}\,\text{s}^{-1} \qquad (2)$$

The calculation of the steady-state emission requires the calculation of the electron steady-state distribution as a function of radius, $N_e(\gamma_e, r)$, given the injection spectrum of equation (2). Earlier treatments of the problem[2-4] assumed this distribution, thereby bypassing the question of the origin of the relativistic electrons. It was nonetheless shown (see, for example, ref. 3) that, assuming the power-law forms $N_e(\gamma_e, r) \propto r^{-n}\gamma_e^{-p}$ and $B(r) \propto r^{-m}$, the superposition of synchrotron self-absorbed components gives a spectrum $F_\nu \propto \nu^{-\alpha_t}$ with

$$\alpha_t = \frac{3m + 5n + 2p(m-1) - 13}{[2n + m(p+2) - 2]} \qquad (3)$$

The exponent α_t can be set to any value (in particular zero) by the appropriate choice of m, n and p. The fact that in a class of objects (the flat-spectrum radio sources) the combination of m, n and p is such that $\alpha \approx 0$ came to be known as 'cosmic conspiracy' (see, for example, ref. 12).

In the present model, the free independent choice of these parameters is not allowed because they are related to the dynamics of energy release near the black hole through the energy transport by neutrons and the resulting secondary-elec-

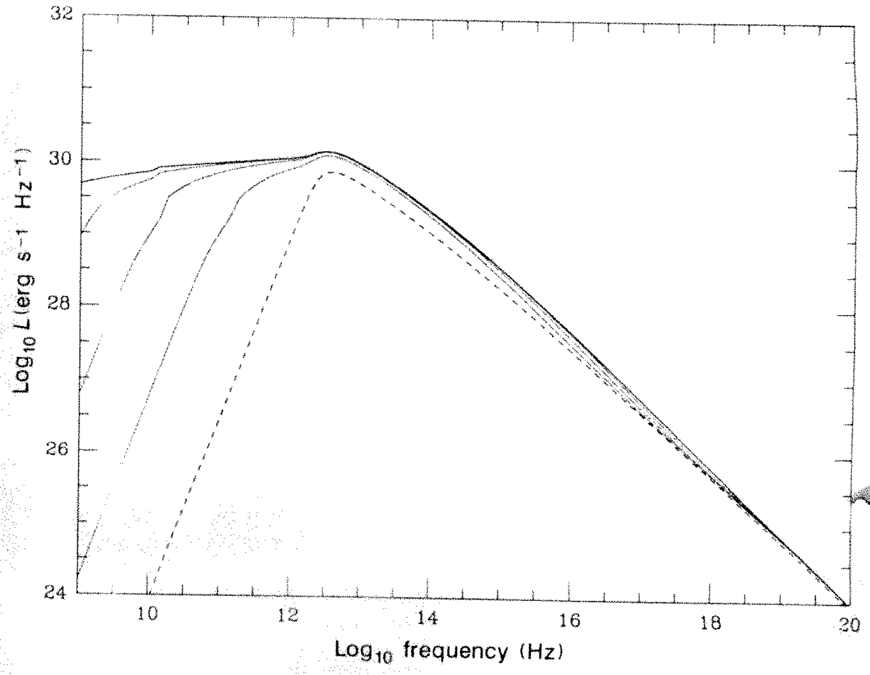

FIG. 1 Logarithmic plot of L_ν against ν for a source with luminosity in neutrons (at $r = r_0$) $L_n = 10^{45}$ erg s^{-1}, magnetic field at $r \leq r_0$, $B_0 = 300$ G, central source size $r_0 = 3 \times 10^{14}$ cm and $\gamma_{p,\max} = 10^5$. The solid line is the final spectrum, that is, the spectrum at infinity. The dashed line shows the spectrum from just the central source, that is, $r \leq r_0$. The dotted lines show the spectrum of the radiation produced inside $r = 10r_0$, $10^2 r_0$ and $10^3 r_0$.

tron production. More importantly, these parameters can be calculated once the electron injection rate as a function of energy and radius, $Q_e(\gamma_e, r)$, is given. The resulting steady-state electron distribution function is then

$$N_e(\gamma_e, r) = \frac{1}{\dot{\gamma}} \int_{\gamma_e}^{\infty} Q_e(\gamma'_e, r)\, d\gamma'_e \tag{4}$$

where $\dot{\gamma}$ is the electron energy loss rate. In the vicinity of an AGN the dominant energy loss processes for relativistic electrons are synchrotron and inverse Compton scattering. For these processes the energy loss rate (in the Thomson approximation) is

$$\dot{\gamma}(r) = \frac{4}{3} \frac{\sigma_T \gamma_e^2}{m_e c} \rho(r) \tag{5}$$

where $\rho(r) = L/4\pi r^2 c + B^2(r)/8\pi$ is the total energy density in photons and magnetic fields. If the magnetic field is in equipartition with the photon energy density (or tied to an outflowing wind), $B(r) \propto 1/r$ and hence $\dot{\gamma}(r) \propto 1/r^2$ for $r \geqslant r_0$, whereas it is assumed to be constant for $r < r_0$.

Under these conditions, equation (4) indicates that for $\gamma > \gamma_{e,b} \approx 60r/c\tau$, the electron distribution function is $N_e(\gamma_e) = A_> \gamma_e^{-q}$, $q = s + 2$, with the normalization $A_>$ independent of the distance r because the geometric dilution factor r^{-2} in $Q_e(\gamma_e, r)$ is cancelled by the corresponding smaller $\dot{\gamma}$ ($\dot{\gamma} \propto r^{-2}$). For $\gamma_e < \gamma_{e,b}$ the electron spectrum is a power law of index 2, $N_e(\gamma_e) = A_< \gamma_e^{-2}$, independent of the details of injection, the factor γ_e^{-2} simply reflecting the energy dependence of the electron loss rate (see, for example, ref. 13), but with a normalization $A_<$ that is r-dependent. The requirement then that these two spectra join at the break energy $\gamma_{e,b}$ (with $\gamma_{e,b} \propto r$) forces the normalization of $N_e(\gamma_e, r)$ for $\gamma < \gamma_{e,b}$ to be $A_< \propto \gamma_{e,b}^{-(q-2)} = \gamma_{e,b}^{-s} \propto r^{-2}$ for $s \approx 2$. It is thus important to note that the radial dependence of $A_<$ is closely tied to the spectral index q at $\gamma > \gamma_{e,b}$ through the matching of the two spectra at $\gamma = \gamma_{e,b}$; the $A_< \propto r^{-2}$ dependence is in fact obtained only if $q \approx 4$ ($s \approx 2$).

The assumption of energy transport by neutrons therefore forces a relativistic-electron distribution that is a power law of index $p = 2$, with normalization $A_< \propto 1/r^2$ (that is, $n = 2$), and as argued $B(r) \propto 1/r$ ($m = 1$) is a reasonable assumption for the magnetic-field radial dependence. This is precisely one of the sets of values of m, n and p that results in $\alpha_t = 0$, thereby producing flat radio spectra in the $\sim 10^9$-$10^{12.5}$ Hz frequency range. (The emission by electrons of energies $\gamma \approx \gamma_{e,b}$ is always optically thin and hence this part of the electron distribution should not be used in equation (3).)

We have performed in detail the calculations qualitatively outlined above, taking into account the exact form of the electron losses and emissivities (employing the Klein–Nishina cross-sections), the full treatment of the synchrotron self-Compton interactions (including iterations until convergence when the inverse-Compton luminosity was higher than that of synchrotron)[14], the precise calculation of the synchrotron emission and absorption coefficients, and the reinjection of e^+e^- pairs as a result of photon–photon absorption of the π^0-decay γ-rays. The detailed calculations have essentially confirmed our qualitative analysis. A sample of such a calculation is given in Fig. 1 where the spectrum of a source with luminosity $L = 10^{45}$ erg s^{-1}, size $r_0 = 3 \times 10^{14}$ cm and $B(r_0) = 300$ G is presented, spanning the range 10^9-10^{20} Hz.

Our model also provides for radio-quiet objects with the introduction of only one more parameter: the density of the ambient accreting matter (or photons) in the vicinity of the 'central engine', the effect of which is to convert the escaping neutrons into protons by means other than their decay, namely, by collisions. Interestingly, the cross-section for these processes increases logarithmically with energy, resulting in shorter mean free paths for the higher-energy neutrons. Thus, when the ambient density becomes larger than a certain value, these collisions preferentially deplete the highest-energy neutrons which are responsible for providing the relativistic electrons at the largest radii and hence at the lowest frequencies; as a result the emission at $\nu \leqslant 10^{12.5}$ Hz is suppressed and the final spectrum is similar to that of a radio-quiet object. Because the neutron mean free path scales like $\dot{m}/\dot{m}_E$ ($\dot{m}_E$ is the Eddington accretion rate) for a simple spherical-accretion model[10], the above arguments indicate that the radio-loud objects should operate at lower values of this parameter than the radio-quiet ones.

The present treatment involves a number of simplifying assumptions that might, at first sight, be objected to on observational grounds. One of them is that of spherical symmetry, although similar spherically symmetric models do exist in the literature[2–4]. Observations do indicate the presence of an unresolved core and linear structures composed of individual components (blobs) of angular sizes of a few milliarcseconds, resolved by very-long-baseline interferometry, that seem to be aligned with the larger scale (arcsec) radio jets. We point out, however, that our models pertain mainly to the unresolved core rather than to these individual 'blobs', which should represent emission from much larger distances. The unresolved core seems to contain most of the flux at frequencies $\gtrsim 5$ GHz and at the same time it is in itself 'flat' down to 5–10 GHz. For example, in the case of NRAO140, extensively analysed in ref. 15, these individual components appear at linear scales ~10–50 pc, that is, more than an order of magnitude larger than those of our models. The spherical model outlined above should in any case be an excellent approximation to a conical jet. Considering the rather isotropic emission of neutrons at the central source, these jets would be expected to have large opening angles ($\gtrsim 1$ sr), at least at scales $\lesssim 1$ pc. This is not as detrimental a feature as it might seem considering the collimated jets observed at larger (arcsec) angular scales, because collimation may in fact be affected by the pressure gradients of the underlying galaxy[16]. Large-opening-angle jets over length scales $\lesssim 1$ pc, which collimate at larger scales ($\gg 1$ pc) may also help resolve the problem of the statistics of unbeamed sources[12]. The apparently superluminally moving 'blobs' could then represent emission from only a small fraction of this 'large angle' jet, namely the fraction that lies in a small angle $\theta \approx \Gamma_{jet}^{-1}$, ($\Gamma_{jet}$ being the jet's bulk Lorentz factor) to the observer's line of sight.

Another simplification pertaining to the observed spectra, the main thrust of the model, is the assumption that the emitting plasma is static. We have also run models relaxing this assumption by including adiabatic losses in the calculations (adding the term $3\dot{r}/r \approx 3c/r$ in $\dot{\gamma}$). In certain cases this modified the emission, yielding slightly inverted spectra ($\alpha_t \approx -0.3$) at the lowest frequencies (~10^9 Hz), which are not in disagreement with the data[1]. A related issue is that of source variability (irrelevant in the present static, steady-state models), which pertains to the proton collision timescale. Simple spherical-accretion scaling indicates densities ~10^4–10^5 cm^{-3}, at distances $\lesssim 1$ pc appropriate for the lowest frequency (GHz) emission, which yield timescales 30–300 yr. The lower figure is in fact consistent with the observed variability of NGC1275 (ref. 17). Faster variability is expected at higher frequencies ($t_{var} \propto \nu^{-3/2}$), as observed. We will address this important issue in detail in a future publication. Finally, we have also neglected the effects of photon–hadron interactions, which may become important for neutrons of Lorentz factors $\gamma_n > 10^5$, as indicated in ref. 18.

Besides the inhomogeneous spherical models[2–4], emission from jet models for these objects also exist[19]; we argue, however, in favour of the spherically symmetric (or conical jet) ones on the basis of their simplicity and the smaller number of free parameters they involve. Even in this more restricted class of models, the present one is significantly more constrained in the allowed values of m, n and p. In particular, the value of p is completely model-independent[13], as it simply reflects the form of the energy losses of relativistic electrons. The value of n does depend on the assumed proton spectrum in the central source ($N_p \propto \gamma_p^{-2}$ being a reasonable assumption); however, the value of n required to produce a flat radio spectrum, namely $n=2$, is obtained only after this proton spectrum has been supplemented by the specifics of the energy transport by neutron escape and subsequent decay (such as the steepening of the proton spectra to γ^{-3}). Finally the choice $B(r)\propto 1/r$ is the most natural one by the arguments already given. It could therefore be argued that the flat spectra of the compact radio sources are an issue not of 'cosmic conspiracy', but rather of 'cosmic necessity', in the sense that their form can result from the simplest, most conservative assumptions, once the presence of relativistic protons in the 'central engine' of these sources is accepted. ☐

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1. Landau, R. *et al.* *Astrophys. J.* **308,** 78–92 (1986).
2. Condon, J. J. & Dressel, L. L. *Astrophys. Lett.* **15,** 203–207 (1973).
3. Marscher, A. P. *Astrophys. J.* **216,** 244–256 (1977).
4. Band, D. L. & Grindlay, J. E. *Astrophys. J.* **308,** 576–591 (1985).
5. Kazanas, D. & Ellison, D. C. *Nature* **319,** 380–382 (1986).
6. Kirk, J. G. & Mastichiadis, A. *Astr. Astrophys.* **213,** 75–79 (1989).
7. De Kerret, H. *et al.* *Phys. Lett.* **B63,** 477–482 (1976).
8. Bell, A. R. *Mon. Not. R. astr. Soc.* **182**, 147–156 (1978).
9. Blandford, R. D. & Ostriker, J. P. *Astrophys. J.* **221,** L29–L32 (1978).
10. Kazanas, D. & Ellison, D. C. *Astrophys. J.* **304,** 178–187 (1986).
11. Sikora, M. *et al.* *Astrophys. J.* **341,** L33–L36 (1989).
12. Phinney, E. S. in *Astrophysics of Active Galaxies and Quasi-Stellar Objects* (ed. Miller, I. S.) 453–496 (University Science Books, Mill Valley, 1985).
13. Kazanas, D. *Astrophys. J.* **287**, 112–115 (1984).
14. Zdziarski, A. A. & Lamb, D. Q. *Astrophys. J.* **309,** L79–L82 (1985).
15. Marcher, A. P. *Astrophys. J.* **334,** 552–559 (1988).
16. Krautter, A., Henriksen, R. N. & Lake, K. *Astrophys. J.* **269,** 81–93 (1983).
17. Backer, D. C. in *Superluminal Radio Sources* (eds Zensus, J. A. & Pearson, T. J.) 76–82 (Cambridge University, 1987).
18. Sikora, M. *et al.* *Astrophys. J.* **320,** L81–L85 (1987).
19. Königl, A. *Astrophys. J.* **243,** 700–709 (1981).

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Variation of low-order acoustic solar oscillations over the solar cycle

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GLOBAL acoustic oscillation modes of the Sun were discovered eleven years ago[1]. The possibility of temporal variations in the oscillation frequencies was suggested by fluctuations in the flux of solar neutrinos[2], and would also be implied by changes in the size of the solar cavity or in the speed of sound within the Sun. Our group has studied solar oscillations for many years[1,3]; over the past decade, a network of three stations deployed at sites that can permit 24-h data collection has provided p-mode (acoustic) spectra of very high quality. Here we present evidence that the frequencies of the lowest-order ($l \leqslant 2$) modes have varied over the period of observation (1977–88) in a manner that is correlated with solar activity (as measured by sunspot number). The frequency variation has a peak-to-peak amplitude of 0.46 ± 0.06 μHz, and could reflect variations in the solar dimensions or in the sound speed in the Sun, which might in turn be due to changes in solar temperature and/or magnetic field.

Detections of changes in the Sun's diameter have been reported[4,5] but they are considered marginal[6]. Luminosity changes[7] seem to have been established only recently[8], but these may be a surface phenomenon rather than necessarily implying changes in the eigenfrequencies. There have been several reports on changes in the frequencies of the low-l modes[9–18], but some confusing conflicts have arisen (compare, for example, ref. 11 with refs 12 and 13).

Here we present some of the first results from a network of three stations situated around the world in such a way that in ideal conditions 24-h data collection is possible. The data reported cover the years 1977–88 and, in more recent years with the increased coverage resulting from multi-station operation, give rise to p-mode spectra of very high quality. We believe that the combination of long, continuous data records, better signal-to-noise ratio and a better window function provided by multi-station operation provide data of the highest statistical accuracy combined with the smallest systematic errors available at present.

The Birmingham group began measurements of the globally averaged velocity of the solar surface in 1974 (ref. 3). Every year since 1975 (except 1979) measurements have been made in Tenerife in collaboration with the Instituto de Astrofisica de Canarias, with increasing coverage through the years. Since 1984, data acquisition has been almost continuous. The instrument used is a resonant scattering spectrometer[19] which uses a reference cell containing potassium vapour as a standard, by which shifts in the potassium Fraunhofer line at 769.9 nm may be measured. Variations in the observer's velocity resulting from the Earth's spin and orbit provide a continuous calibration of velocity. Timing is provided to a precision of the order of one second and therefore contributes fractional errors of the order of 10^{-7} for each data set. A second station using an updated instrument operating on principles similar to that in Tenerife was deployed in 1981 at Haleakala in Hawaii and was used during the summers until 1986, when a degree of automation

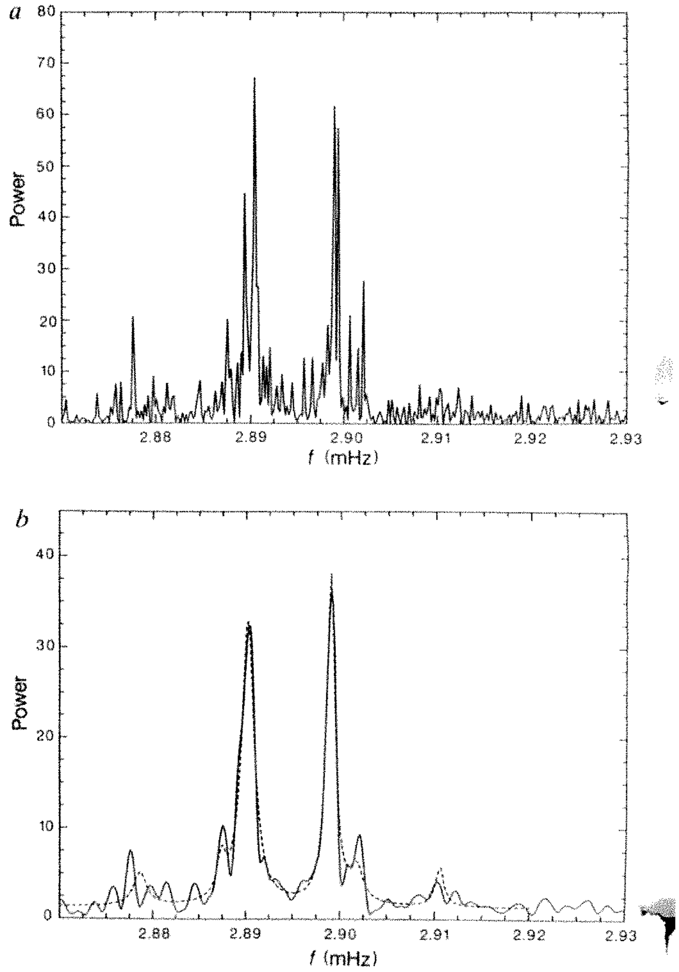

FIG. 1 Portion of a typical power spectrum, in *a*, unsmoothed and *b*, smoothed forms, and fitted by a least-squares technique to a function consisting of two lorentzian peaks of independent height and width with associated sidelobes ±11.57 μHz away on a flat background.

TABLE 1 Composition of power spectra

Year	Julian dates 2440000+	Station	% Time
1977	3336-3379	T	17
1978	3720-3731	T	28
	3745-3760	T	26
1980	4438-4468	T	32
1981	4761-4824	HT	54
1982	5134-5192	HT	48
1983	5507-5569	HT	48
1984	5870-5932	HT	48
1985	6153-6214	T	18
	6215-6276	T	19
1986	6598-6640	HT	23
	6690-6752	TA	23
1987	6978-7040	HTA	44
1988	7237-7299	HTA	33
1988	7350-7412	HTA	61
1988	7431-7492	HTA	30

H, Haleakala, Hawaiian Islands; T, Izana, Tenerife; A, Carnarvon, Western Australia; data were combined as indicated. % time is the percentage of the total observing time in the given interval.

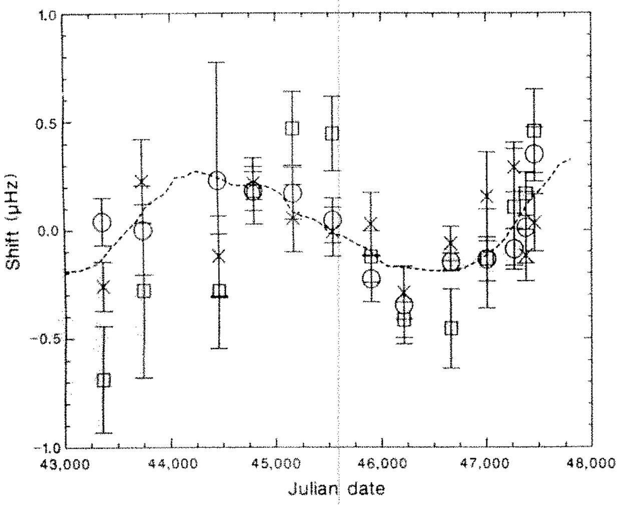

FIG. 2 Variation between 1977 and 1988 of the mean shift for modes with $l=0$, $n=17$-24 (circles), $l=1$, $n=17$-24 (crosses) and $l=2$, $n=16$-23 (squares). The dashed line corresponds to the best linear fit of the shifts to the sunspot number, smoothed over 3 months (Fig. 3).

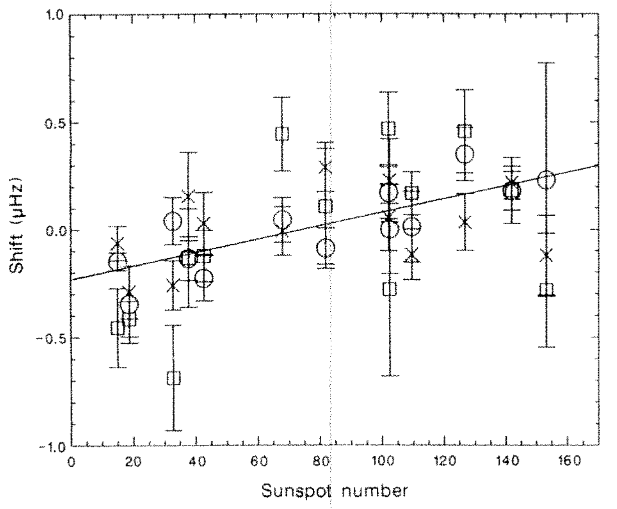

FIG. 3 Correlation between 1977 and 1988 of the mean shift of modes for $l=0$, 1 and 2 with the smoothed sunspot number[23].

allowed more extensive data taking. A third station, incorporating further levels of automation, was deployed in August 1985 at Carnarvon, Western Australia. Despite instrument failures and logistical problems it has proved possible to collect reasonably well filled data stretches covering two-month periods for several years[20]. These data form the basis for the analysis presented below and for the work on linewidth discussed elsewhere[21].

Velocity residuals with 42-s spacing, were formed from the raw data by removing the daily trend and substituting zeros for missing points. Power spectra were obtained using a standard fast-Fourier-transform routine. The composition of the spectra used is summarized in Table 1. It should be noted that the signal-to-noise and fill factor were poorer in the early years.

The frequencies of modes were obtained by least-squares fitting to the power spectra, as illustrated for example in Fig. 1. The sidelobes introduced by the day-night cycle, at 11.57 μHz above and below each genuine peak, were taken into account in the fitting function. These sidelobes are very nearly eliminated in good three-station spectra. Tests were carried out for good-quality one-station data which demonstrate that even with the aliases, similar results (but with larger errors) are obtained. Because an unsmoothed power spectrum of modes with lifetimes shorter than, but comparable to, the observation time has a ragged 'spiky' appearance which makes it difficult to decide on the true frequency of the modes, the spectra were first smoothed. A non-distorting function[22] was used, with a full width at half maximum of $n/3$ frequency bins for smoothing over n bins: smoothing was performed over several bins increasing from 5 to 49 over the frequency range 2-3.8 mHz, the degree of smoothing being chosen so as to remove the spurious internal structure of the peaks without seriously increasing the measured linewidth. The applicability of the fitting technique is limited by the decreasing linewidth below about 2.2 mHz: above about 3.7 mHz the modes are poorly resolved because of the increased linewidth.

A selected set of strong modes ($n=17$-24) was used in the analysis. The mean frequency of each mode over all the spectra was calculated, allowing the shift of each mode from its mean to be evaluated for each spectrum. The mean shift over all modes of a given l for a given year is thence obtained; those for $l=0$, 1 and 2 in the central region of the spectrum are plotted in Fig. 2 (error bars are standard deviation of the mean). The dashed line corresponds to the best linear fit of shift to sunspot number[23], R_z, smoothed over three months, using the correlation shown in Fig. 3. The correlation coefficient for the 39 points is 0.69.

The acoustic eigenfrequencies considered here correspond to $n \approx 20$ and $l=0$, 1 and 2. If we restrict the discussion to the radial modes we can consider them to be the result of plane standing waves trapped in a cavity of radius $2R_0$ and propagating with a velocity $v(R)$, the local value of which is given approximately by $v=\sqrt{(\gamma kT/m)}$ in the absence of strong magnetic fields (where γ is the adiabatic index and m is the mean molecular weight). The total travel time t is given by

$$t=2\int_0^{R_0}\frac{\mathrm{d}r}{v(R)}$$

and is strongly weighted towards values at the surface: $v(R_0)=7\ \mathrm{km\ s^{-1}}$ compared with $v(0)\approx 500\ \mathrm{km\ s^{-1}}$. The travel time t is roughly equal to the inverse spacing of the spectrum of eigenfrequencies[1] (135.6 μHz), that is, $t\approx 7{,}370$ s.

The observed peak-to-peak variation of the frequencies, 0.46 ± 0.06 μHz, corresponds to a change in the total travel time of ~1 s. The travel time reaches a maximum at the time of the minimum solar activity. This change could be the result of a change in cavity dimension and/or the velocity of sound in the interior. One of the possible first-order contributions to the variation in sound velocity is variations in temperature T. Sufficiently large changes in γ and m, and in second-order propagation delays due to turbulent motions[24], are improbable.

Changes in the strong internal solar magnetic fields, such as have been postulated[25] and considered in the context of the eigenfrequencies of the Sun[26-29], are also possible candidates. □

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1. Claverie, A., Isaak, G. R., McLeod, C. P., van der Raay, H. B. & Roca Cortes, T. *Nature* **282**, 591–594 (1979).
2. Sakurai, K. *Solar Phys.* **74**, 35–41 (1981).
3. Brookes, J. R., Isaak, G. R. & van der Raay, H. B. *Nature* **259**, 92–95 (1976).
4. Gilliland, R. L. *Astrophys. J.* **248**, 1144–1155 (1981).
5. Delache, P., Laclare, F. & Sadsaoud, H. *Nature* **317**, 416–418 (1985).
6. JOSO Working Group 5 Meeting, November 1989, Rome.
7. Gough, D. O. *Nature* **288**, 639–640 (1980).
8. Willson, R. C. & Hudson, H. S. *Nature* **332**, 810–813 (1988).
9. van der Raay, H. B. in *Theoretical Problems in Stellar Stability and Oscillations* (eds Noels, A. & Gabriel, M.) 215–219 (Université de Liege, Cointe-Ougree, 1984).
10. Isaak, G. R. *et al. Adv. Helio- Asteroseismology IAU Symp.* **123**, 201–204 (1988).
11. Jefferies, S. M., Palle, P. L., van der Raay, H. B., Regulo, C. & Roca Cortes, T. *Nature* **333**, 646–649 (1988).
12. Palle, P. L., Regulo, C. & Roca Cortes, T. *Seismology of the Sun and Sun-like Stars: ESA SP-286*, 285–289 (European Space Agency, Paris, 1988).
13. Palle, P. L., Regulo, C. & Roca Cortes, T. *Astr. Astrophys.* **224**, 253–258 (1989).
14. Woodard, M. F. & Noyes, R. W. *Nature* **318**, 449–450 (1985).
15. Woodard, M. F. & Noyes, R. W. *Seismology of the Sun and the Distant Stars* (ed. Gough, D. O.) 303–304 (Reidel, Dordrecht, 1986).
16. Woodard, M. F. & Noyes, R. W. *Adv. Helio- Asteroseismology IAU Symp.* **123**, 197–200 (1988).
17. Fossat, E., Gelly, B., Grec, G. & Pomerantz, M. A. *Astr. Astrophys.* **177**, L47–L48 (1987).
18. Gelly, B., Fossat, E. & Grec, G. *Astr. Astrophys.* **200**, L29–L31 (1988).
19. Brookes, J. R., Isaak, G. R. & van der Raay, H. B. *Mon. Not. R. astr. Soc.* **185**, 1–17 (1978).
20. Aindow, A. *et al. Seismology of the Sun and Sun-like Stars: ESA SP-286*, 157–160 (European Space Agency, Paris, 1988).
21. Elsworth, Y. P. *et al. Mon. Not. astr. Soc.* **242**, 135–140 (1990).
22. Pollard, J. H. *A Handbook of Numerical and Statistical Techniques* 126 (Cambridge University Press, 1977).
23. *Solar Geophysical Data, July 1989; No. 539, part I* (NOAA, Boulder, Colorado, 1989).
24. Brown, T. M. *Science* **226**, 687–689 (1984).
25. Dicke, R. H. *Solar Phys.* **78**, 3–16 (1982).
26. Isaak, G. R. *Nature* **296**, 130–131 (1982).
27. Dicke, R. H. *Nature* **300**, 693–697 (1982).
28. Gough, D. O. *Nature* **298**, 350–354 (1982).
29. Roberts, B. & Campbell, W. R. *Nature* **323**, 603–605 (1986).

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Superconductivity in the layered compound Li_xNbO_2

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SEVERAL classes of copper oxide compounds are high-temperature superconductors[1-3]; the highest known transition temperature (T_c) is 122 K for $Tl_2Ba_2Ca_2Cu_3O_{11}$ (ref. 4). Superconductivity has also been observed in oxides containing early transition metals, but the superconducting transition temperatures are substantially lower. (The highest known T_c for an early transition metal oxide is 13.7 K, for the spinel compound $LiTi_2O_4$ (ref. 5).) Whereas all of the copper oxide superconductors have very anisotropic structures, the superconducting oxides of early transition metals discovered up to now have three-dimensional structures. Here we report the discovery of a new class of superconductors, Li_xNbO_2, with layered structures. At low applied magnetic fields, the magnetic susceptibility greatly decreases to diamagnetic values below 5.5 K for $Li_{0.45}NbO_2$ and below 5.0 K for $Li_{0.50}NbO_2$; this transition indicates the onset of superconductivity. In this first example of superconductivity in a layered early transition metal oxide, it is interesting to note that the layering has not increased T_c to new levels for early transition metal oxides.

The reduced lithium niobate, $LiNbO_2$, has a layered structure analogous to that of MoS_2. As shown in Fig. 1, the lithium ions occupy octahedral holes between sheets of edge-sharing NbO_6 trigonal prisms[6]. The only study of the deintercalation chemistry of $LiNbO_2$ is by Kumada *et al.*[7] who recently reported preparation of Li_xNbO_2 ($0.51 < x < 0.93$) by chemical and electrochemical means. For all materials prepared, they observed temperature-independent paramagnetism between 80–300 K. Owing to the trigonal prismatic coordination of the niobium atoms in $LiNbO_2$, the conduction band, which is derived mostly from d_{z^2} orbitals, is narrow[8]. Because this band is completely filled for stoichiometric $LiNbO_2$ (Nb(III), d^2), this compound is expected to be semiconducting. Partial removal of lithium atoms results in oxidation of the niobium atoms and an increase in the density of states at the Fermi level as a result of the loss of electrons from the d_{z^2} band. Kumada *et al.* observed an increase in the magnetic susceptibility with decreasing x, consistent with an increase in the density of states. Removal of all the lithium atoms would lead to a half-filled d_{z^2} band (Nb(IV), d^1), and a high density of states at the Fermi level. BCS-type superconductivity is often observed when the density of states at the Fermi level is high[9]. Although we do not observe superconductivity in $LiNbO_2$ down to 2 K, partial oxidation of this material by removal of lithium atoms leads to the onset of superconductivity.

The burgundy-red powder, $LiNbO_2$, was prepared by the reaction of Li_3NbO_4 and NbO in evacuated fused-silica tubes at 1,050 °C, as in ref. 7. Pressed pellets of the reaction mixture were placed in alumina boats before sealing to minimize reaction with the quartz. Analysis of the powder X-ray diffraction pattern revealed that the product was predominantly $LiNbO_2$ with only trace amounts of the impurity $LiNbO_3$. Hexagonal lattice parameters $a = 2.9063$ (6) Å and $c = 10.447$ (2) Å were derived from the powder diffraction data using silicon as an internal standard. The lithium content was determined by atomic absorption analyses. The product was slightly non-stoichiometric in lithium, $Li_{0.93}NbO_2$. Partial oxidation of $Li_{0.93}NbO_2$ was carried out by two methods: reaction with bromine and reaction with nitrosyl

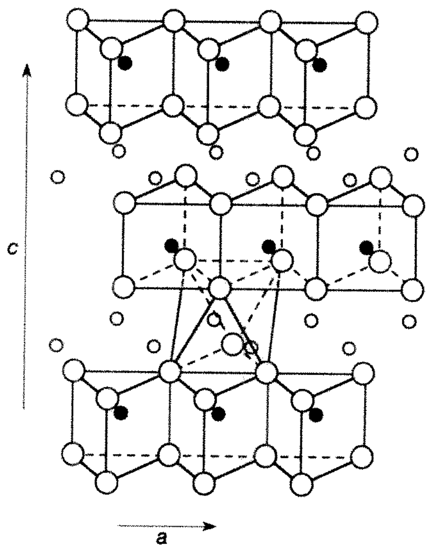

FIG. 1 Schematic of the structure of $LiNbO_2$. The layered structure consists of a hexagonal array of niobium atoms (solid circles) sandwiched between arrays of hexagonally ordered oxygen atoms (large open circles). The coordination about the niobium atoms is trigonal prismatic. The lithium atoms (small open circles) are in octahedral sites between the oxygen–niobium–oxygen layers. One row of niobium atoms and two rows of lithium atoms are shown in each of their respective layers. Sufficient oxygen atoms are shown to indicate the coordination spheres.

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tetrafluoroborate. $Li_{0.50}NbO_2$ was prepared by heating to reflux 1.7 g of $Li_{0.93}NbO_2$ powder in 10 ml of an acetonitrile solution that was 0.3M in Br_2 for 3 days. $Li_{0.45}NbO_2$ was prepared by stirring a 70-ml slurry of 0.97 g of $Li_{0.93}NbO_2$ powder and 1.7 g of $NOBF_4$ in acetonitrile for 10 days. For both reactions, the resultant grey powder was filtered, washed with acetonitrile and ethanol, and dried. Analyses of the X-ray powder diffraction patterns revealed little change from the hexagonal starting material; the pattern obtained for $Li_{0.50}NbO_2$ is shown in Fig. 2*a*. The refined lattice parameters for $Li_{0.50}NbO_2$ were $a = 2.919$ (1) Å and $c = 10.453$ (4) Å, and those for $Li_{0.45}NbO_2$ were $a = 2.9227$ (9) Å and $c = 10.455$ (5) Å.

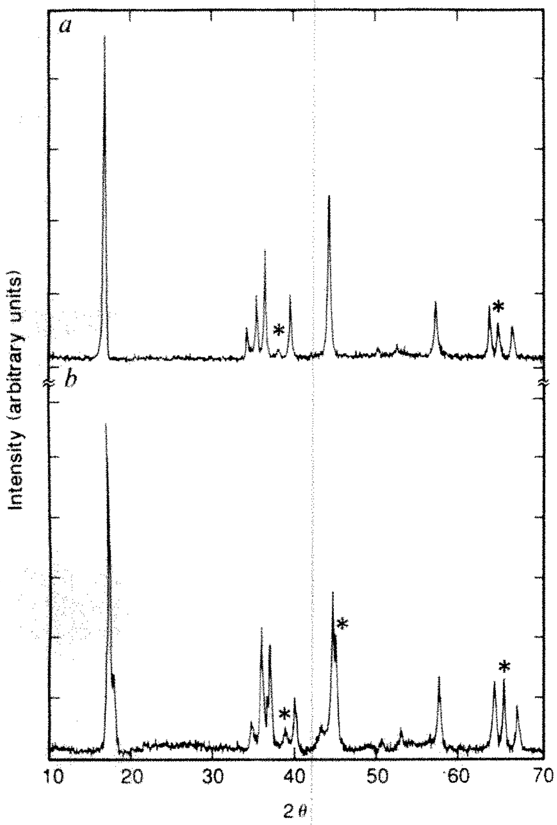

FIG. 2 X-ray powder diffraction patterns of $Li_{0.50}NbO_2$ *a*, before and *b*, after annealing at 300 °C. Peaks due to the aluminium sample holder are designated with an asterisk.

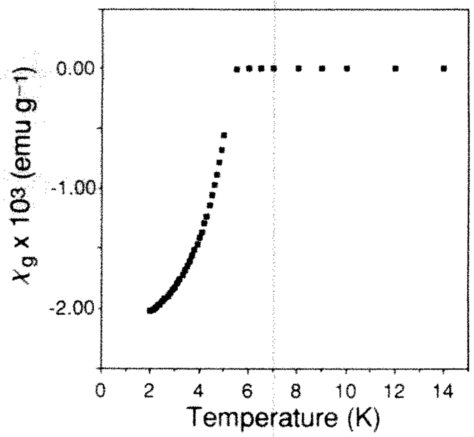

FIG. 3 Meissner effect of $Li_{0.45}NbO_2$ prepared by deintercalation of $LiNbO_2$ with $NOBF_4$. Field = 20.33 G.

The magnetic properties of $Li_{0.50}NbO_2$ and $Li_{0.45}NbO_2$ were measured with a Quantum Design SQUID (superconducting quantum interference device) by cooling in fields of 12.10 G and 20.33 G, respectively. The results for $Li_{0.45}NbO_2$ are shown in Fig. 3. Similar behaviour was observed for $Li_{0.50}NbO_2$. Above ~6 K both samples behave as temperature-independent paramagnets with very small moments. For $Li_{0.45}NbO_2$, the susceptibility drops steeply to diamagnetic values below 5.5 K; this transition indicates the onset of superconductivity. The gram susceptibility at 2 K is -2.02×10^{-3} e.m.u. g^{-1}. Assuming a density of 6.0 g cm^{-3} and ignoring demagnetization corrections, this corresponds to a Meissner effect of 15%. For $Li_{0.50}NbO_2$, T_c onset occurs at the slightly lower temperature of 5 K and the Meissner effect is considerably less. The starting material, $Li_{0.93}NbO_2$, and a sample of $Li_{0.62}NbO_2$ prepared with less Br_2 so as to yield a larger value of x did not show diamagnetism down to 2 K. These results show that trace amounts of Nb metal, which has a T_c of 9 K, are not present as an impurity in the samples. The large Meissner effect for $Li_{0.45}NbO_2$ and the variation of T_c with lithium content provide strong evidence that, when x decreases below a critical value, Li_xNbO_2 becomes a bulk superconductor.

It is useful to compare these results for the oxide with those obtained for Li_xNbS_2, which has a similar structure (ref. 10). For values of x between 0 and 0.4, the superconducting transition temperature for the disulphide varies between 2 and 6.3 K. But above $x \approx 0.40$, superconductivity has not been observed down to 2 K. For a comparable lithium content, we observe that T_c is substantially higher for the dioxides than for the disulphides. We therefore expect T_c will rise above values seen in the disulphides as the lithium content is reduced further. Unfortunately, with the reagents we used, it is not possible to oxidize Li_xNbO_2 further. We are now investigating different chemical and electrochemical means of reducing the lithium content below $x = 0.45$ so that we can prepare the entire series of Li_xNbO_2 materials and study the relationship between composition and superconductivity.

We have been unable to obtain reliable resistivity data on our samples. Although Kumada *et al.* report resistivity data obtained from pressed powders, we do not know whether the semiconducting behaviour that they observed was due to the sample or to grain-boundary resistances. Furthermore, it was also not possible to sinter the samples to get better contact between the grains. When $Li_{0.50}NbO_2$ was heated at 300 °C in an evacuated fused-silica tube for three days, a subtle structural transformation occurred and superconductivity was no longer observed. The X-ray powder diffraction patterns before and after annealing are shown in Fig. 2. A splitting of the most intense peak, indexed as the 002 line in the hexagonal unit cell, indicates lowering of symmetry. Also, the relative intensities of the 004, 100 and 101 lines changed. Differential thermal analysis of the reaction confirmed a structural transformation. There is a strong exotherm beginning about 130 °C and ending at 280 °C indicative of a phase transition; decomposition of this phase occurs near 480 °C. A similar effect was observed on annealing of $Li_{0.50}VO_2$, which converts to the LiV_2O_4 spinel phase at 300 °C (ref. 11). We are investigating the exact nature of the structural transformation and the reason for the loss of superconductivity.

The discovery of superconductivity in these materials is significant for a number of reasons. Previously all of the known superconducting niobium oxides contained niobium that was in a low formal oxidation state. In $Li_{0.45}NbO_2$ the formal oxidation state of niobium is +3.55. Furthermore, we have seen superconductivity in a layered oxide containing an early transition metal; as the superconducting copper oxides also have layered structures, a comparison of the properties of niobium oxide superconductors with those of the high-T_c copper oxide materials will help clarify the mechanism of superconductivity in layered oxides. ☐

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1. Bednorz, J. G. & Müller, K. A. *Z. Phys.* **B64,** 189–193 (1986).
2. Wu, M. K. *et al. Phys Rev. Lett.* **58,** 908–910 (1987).
3. Sheng, Z. Z. & Herman, A. M. *Nature* **332,** 55–58 (1988).
4. Parkin, S. S. P. *et al. Phys. Rev. Lett.* **60,** 2539–2542 (1988).
5. Johnston, D. C. *et al. Mater. Res. Bull.* **8,** 777–784 (1973).
6. Meyer, G. & Hoppe, R. *J. less-common Metals* **46,** 55–65 (1976).
7. Kumada, N. *et al. J. Solid St. Chem.* **73,** 33–39 (1988).
8. Burdett, J. K. & Hughbanks, T. *Inorg. Chem.* **24,** 1741–1750 (1985).
9. Bardeen, J., Cooper, L. N. & Schrieffer, J. R. *Phys. Rev.* **B106,** 162–164 (1957); *Phys. Rev.* **B108,** 1175–1204 (1957).
10. McEwen, C. S. & Sienko, M. J. *Rev. chim. Miner.* **19,** 309–316 (1982).
11. de Picciotto, L. A. & Thackeray, M. M. *Mater. Res. Bull.* **20,** 187–195 (1985).

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Manufacture of bulk superconducting $YBa_2Cu_3O_{7-\delta}$ by a continuous process

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THE high critical current densities (J_cs) measured in thin films and within grains of the high-transition-temperature (high-T_c) oxide superconductors indicate that low J_c should not prevent the use of high-T_c materials in high-current devices[1]. Indeed, a J_c of $\sim 7.5\times10^4$ A cm^{-2} has been obtained in highly textured bulk $YBa_2Cu_3O_7$ ('123') at 0 T, processed by a melt-textured[2] or liquid-phase[3] growth technique. Unfortunately, textured materials with this improved J_c comprised only a very small portion (<1 cm) of the bulk precursor, which had random grain orientations on a larger scale. For high-current applications, one needs bulk materials fabricated in usable shapes of large dimensions with controlled grain orientation to carry a high total current, not just a high current density. Here we report the development of a continuous process for fabricating large bulk superconductors with a pre-determined grain orientation. A bar of the 123 compound with dimensions $5\times0.5\times\sim0.3$ cm with excellent grain alignment (*a-b* plane along the longest sample dimension) has been fabricated continuously. The bulk 123 thus obtained has magnetically determined J_cs of $\sim3\times10^4$ and 1.2×10^4 A cm^{-2} at 0 and 1 T, respectively and transport J_cs of 2×10^4, 1.1×10^4 and 7.5×10^3 A cm^2 at 0, 0.54 and 0.83 T.

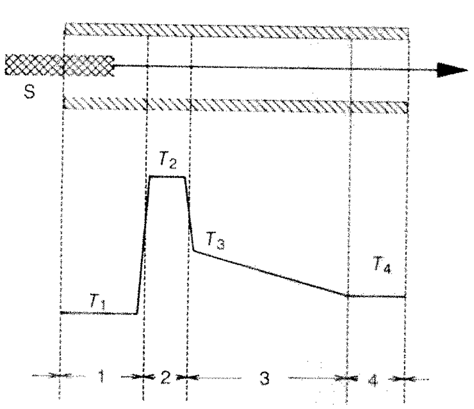

FIG. 1 The schematic of the continuous process for step (3) in the preparation of a bulk 123 sample (S). The temperatures are: $T_1 = 500 \pm 425$ °C, $T_2 = 1{,}150 \pm 50$ °C, $T_3 = 1{,}035 \pm 15$ °C and $T_4 = 925 \pm 25$ °C. The times spent in different zones are: 1; not critical; 2, 10 ± 5 min; 3, 100 ± 50 h; 4, 20 ± 5 h. The peritectic temperature is ~1,050 °C.

The limited J_c in bulk high-T_c superconductors has been attributed[4–9] largely to 'weak links' arising from the presence of one or more of the following: (1) high-angle grain boundaries; (2) secondary phases such as Y_2BaCuO_5, $BaCuO_2$ and CuO; (3) porosity; (4) local or global deficiency of oxygen; (5) microcracks; (6) the electrical anisotropy. To overcome some of these problems, various melt-texturing techniques have been used.

As a 123 bulk precursor cools from a single-phase liquid region to ~1,050 °C it undergoes the transition (Y_2O_3 + liquid) → Y_2BaCuO_5 + liquid) → (123 + liquid of composition $BaCuO_2$ + CuO) (refs 10, 11). Near ~1,050 °C, 123 forms through a peritectic solidification according to Y_2BaCuO_5 + liquid ($3BaCuO_2 + 2CuO$) → $2YBa_2Cu_3O_{6.5}$. Heating the material above the solidus temperature (1,020–1,200 °C) helps to dissolve the impurity phases, which are precipitated in the grain boundaries. Also, crystal grains grow more easily in a liquid solution. Therefore, the reported methods[2,3,12,13] for making 123 that is capable of carrying a high J_c consist of rapidly heating 123 above the solidus temperature (or even above the melting point) and slowly cooling through the peritectic temperature. This has been achieved using a prescribed temperature schedule, that is, different specified rates of temperature change (dT/dt) for various temperature regions. The morphology of the grains obtained depends critically on the temperature schedule[2,14], although in a complex manner that remains to be clarified. Some workers[2] have obtained ordered grain growth by directional solidification using a temperature gradient along the length of the material, dT/dx, whereas others[3] have approached the problem without using temperature gradients. After completion of our work, it came to our attention that McGinn *et al.*[15,16] have carried out a texture processing of bulk 123 by zone melting. They did not use a prescribed temperature gradient and achieved only small domains of aligned grains, which had a magnetically determined J_c of $\sim1.8\times10^4$ A cm^{-2} at 2 T and a transport J_c of ~500 A cm^{-2} at 0.5 T. To allow the continuous growth of large samples of bulk 123 that are capable of carrying a high J_c and to help remove impurities, we move the 123 bulk precursor through an open tubular furnace having a pre-set temperature profile that includes a narrow hot zone (Fig. 1). We vary both the speed, dx/dt, and the gradient, dT/dx, to give the desired rate of temperature change, dT/dt.

A bar of dimensions $5.2\times0.5\times0.3$ cm was sintered from a 123 fine-powder precursor at 940–960 °C for 24 h in an oxygen atmosphere to obtain ≥90% of the theoretical density, 6.3 g cm^{-3}. The bar was heated to ~1,100 °C very rapidly for a short time before it was cooled rapidly to near the peritectic temperature (~1,050 °C). The sample was then allowed to undergo peritectic solidification very slowly. Because of the narrow hot zone, zone refining occurs along the axis of travel depositing Y_2BaCuO_5 at one end of some of the samples. Pieces from different parts of the bar were oxygenated at 500 °C for 24–48 h. Figure 2 shows representative scanning electron micrographs of the longitudinal and transverse fracture faces of the pieces. There is excellent grain alignment throughout the bar. The X-ray diffraction patterns in Fig. 2 clearly show that the *a–b* plane coincides with the longitudinal fracture face along the direction of travel and that all of these planes in the sample are parallel to each other. The electrical and magnetic susceptibility measurements (Fig. 3) exhibit sharp transitions, demonstrating the high superconducting quality of the sample. The small Meissner signal determined during field cooling compared with that obtained during zero-field cooling (Fig. 3) indicates the strong flux pinning existing in those samples. According to the Bean formula ($J_c = 20\,\Delta M/d$ where ΔM is the difference in the remnant moments of the sample during the increasing and decreasing field sweeps, and d is the sample dimension

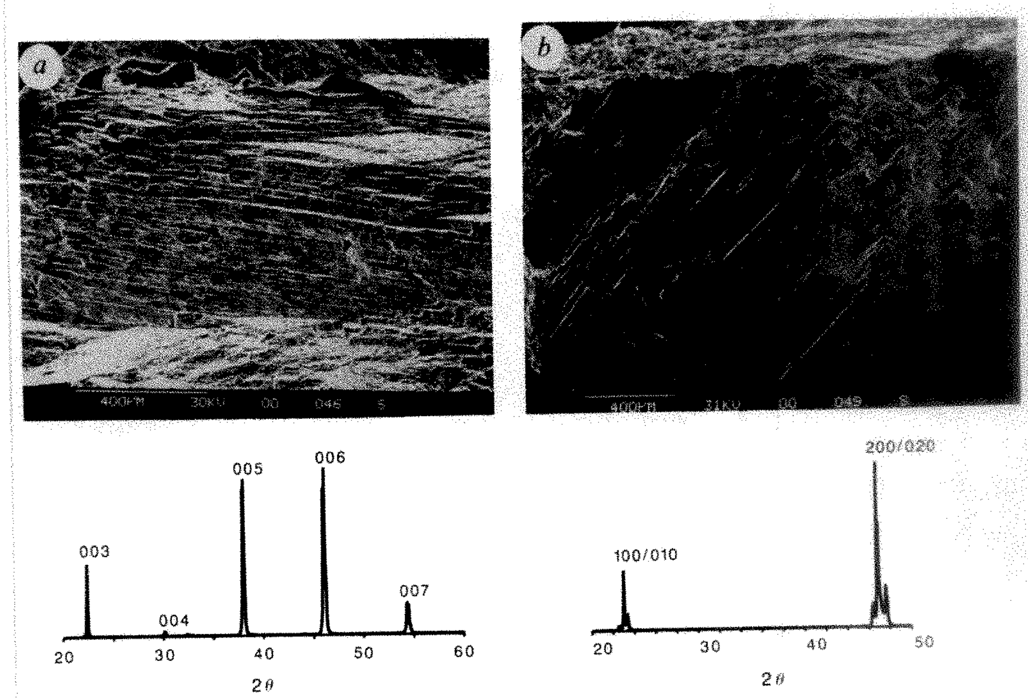

FIG. 2 Representative scanning electron micrographs of the *a*, longitudinal and *b*, transverse fracture surfaces of the bar of 123 compound, together with their X-ray diffraction patterns.

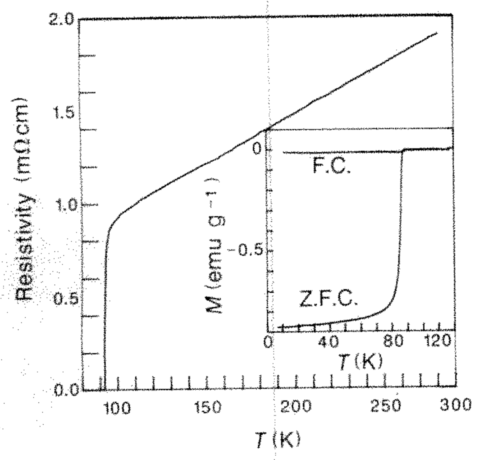

FIG. 3 Resistive and magnetic transitions of the continuously processed 123 (F.C., field cooling; Z.F.C., zero-field cooling).

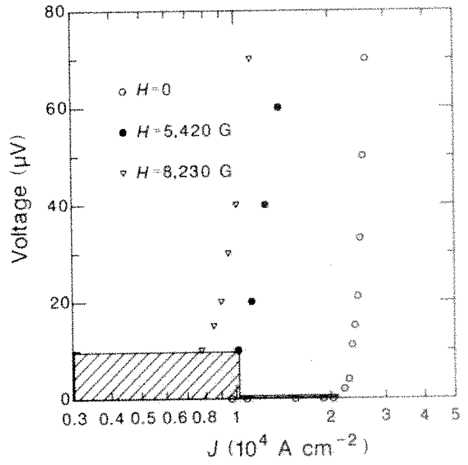

FIG. 4 Voltage as a function of current density at different magnetic fields (perpendicular to J_c) at 77 K. The resolution was 0.2 μV and 10 μV, respectively, at a magnetic field, *H*, of 0 and >0.4 T, in contrast to the 50 μV previously achieved[3].

perpendicular to the field) the J_c of a sample of dimensions $0.216 \times 0.165 \times 0.165$ cm (that is, $d = 0.165$) is 3×10^4 and 1.2×10^4 at 0 and 1 T, respectively. At 77 K and with the magnetic field perpendicular to J_c the transport J_c (Fig. 4) in samples of dimensions $\sim 1 \times 0.04 \times 0.05$ cm was $\sim 2 \times 10^4$ A cm^{-2} at 0 T, 1.1×10^4 A cm^{-2} at 0.54 T and 7.5×10^3 A cm^{-2} at 0.82 T. When the magnetic field is parallel to J_c, $J_c \sim 1.4 \times 10^4$ A cm^{-2} at 0.82 T, suggesting that weak links are not a serious problem in materials processed in this way. Transport J_cs as low as 3×10^3 A cm^{-2} at 0 T, however, were detected in one piece of the sample. We attribute the difference to chemical inhomogeneity. For example, the oxygen content (determined by a Raman microprobe) varied from 6.92 to 6.87. We believe that adjusting the processing parameters should give a material with a higher, more uniform transport J_c.

Because of the continuous nature of the process, desired sizes of practical forms (such as plates, rods, wires, ribbons and even thick films) could be made from highly textured bulk superconductors having high J_cs. The processing of the bulk 123 can be divided into four steps: (1) synthesis of powder precursor; (2) sintering of bulk precursor; (3) melt-textured growth; (4) oxygenation. By adopting the proper temperature gradients and speeds in different zones of the furnace, steps (2)–(4) can be combined into a single continuous process. The processing temperature, particularly in step (3), can be lowered by using low-melting flux consisting of $BaCuO_2$ and/or CuO, a more reducing atmosphere, or halogen[17]. This is especially crucial for wires or ribbons of superconductors having metal cladding such as a silver sheath. At present, the process is slow but by varying the processing parameters and possibly the phase diagram of Y–Ba–Cu–O through doping, we expect to improve the speed of the grain growth. □

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1. Chu, C. W. *Science* **245**, 111–112 (1989).
2. Jin, S. *et al. Appl. Phys. Lett.* **42**, 2074–2076 (1988).
3. Salama, K., Selvamanickam, V., Gao, L. & Sun, K. *Appl. Phys. Lett.* **54**, 2352–2354 (1989).
4. Dinger, T. R., Worthington, T. K., Gallagher, W. J. & Sandstrom, R. L., *Phys. Rev. Lett.* **58**, 2687–2690 (1987).
5. Nakahara, S., Fisanick, G. J., Yan, M. F., van Dover, R. B. & Boone, T. *J. Cryst. Growth* **85**, 639–650 (1987).

6. McGuire, T. R. *et al. Phys. Rev.* **B36,** 4032–4035 (1987).
7. Tozer, S. W., Kleinsasser, A. W., Penney, T., Kaiser, D. & Holtzberg, F. *Phys. Rev. Lett.* **59,** 1768–1771 (1987).
8. O'Bryan, H. M. & Gallagher, P. K. *Adv. Ceram. Mater.* **2,** 640 (1987).
9. Ekin, J. W. *et al. J. appl. Phys.* **62,** 4821–2828 (1987).
10. Aselage, T. & Keefer, K. *J. Mater. Res.* **3,** 279–291 (1988).
11. Murakami, M., Morita, M., Doi, K., Miyamoto, K. & Hamada, H. *Jap. J. appl. Phys.* **28,** L399–L401 (1989).
12. Murakami, M., Morita, M., Doi, K. & Miyamoto, K. *Jap. J. appl. Phys.* **28,** 1189–1194 (1989).
13. Alford, N. M. *et al. J. appl. Phys.* **66,** 5930–5934 (1989).
14. McGinn, P. J., Black, M. A. & Valenznek, A. *Physica* C**156,** 57–61 (1988).
15. McGinn, P. J., Chen, W. & Black, M. A. *Physica* C**161,** 198–203 (1989).
16. McGinn, P. J., Chen, W., Zhu, N., Balachandran, U. & Lanagam, M. T. *Physica* C**165,** 480–484 (1989).
17. Radousky *et al.* Preprint (Lawrence Livermore National Laboratory).

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Surface-adsorbed free radicals observed by positive-muon avoided-level-crossing resonance

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HETEROGENEOUSLY catalysed reactions usually proceed at outer or inner surfaces of materials that have large and often non-uniform surface area. Many intermediates of such chemical transformations are short-lived and escape direct detection; such transient species may include free radicals. The structure, adsorption and surface dynamics of diamagnetic intermediates have been well studied by thermodynamic or spectroscopic methods, but paramagnetic species (that is, radicals) recombine at catalytically relevant temperatures so that they are never present in sufficiently high concentrations to allow their observation by these techniques. The otherwise powerful laser methods are also hampered by the non-transparent nature of heterogeneous systems. Here we demonstrate for the model case of cyclohexadienyl radicals on silica that a novel technique, positive-muon avoided-level-crossing spin resonance, which uses positive muons as spin probes, is sufficiently sensitive to allow the detection and study of radicals under such constrained conditions. The technique can detect radical concentrations many orders of magnitude less than can be studied by conventional magnetic spectroscopy.

Spin-polarized energetic positive muons injected into matter are used routinely to sense magnetic interaction phenomena in various environments[1]. As they thermalize in the sample a certain fraction form muonium atoms ($\mathrm{Mu} \equiv \mu^+e^-$) by electron capture near thermal energy. Mu behaves as a very light isotope of hydrogen ($m_{\mathrm{mu}} \approx \frac{1}{9} m_{\mathrm{H}}$) and undergoes similar chemical reactions. In particular, it adds to unsaturated bonds to form muonated free radicals in which the muon is a spin-polarized substitute for a normal hydrogen nucleus[2]; for example, addition to benzene leads to the muonated cyclohexadienyl radical C_6H_6Mu. A single-particle-counting technique that takes advantage of the asymmetry of the muon decay ($\tau_\mu = 2.2\ \mu s$, $\mu^+ \rightarrow e^+ + \bar{\nu}_\mu + \nu_e$, the positron being emitted preferentially along the instantaneous muon spin axis) reveals the evolution of spin polarization. In the conventional variant of the experimental technique, with a magnetic field transverse to the initial spin polarization[1], temporal variations in the arrival rate of decay positrons in a given direction show the precession frequencies of the muons; the signal is analogous to a free-induction decay in pulsed magnetic resonance. This method has been used to study the radiolytic formation process and the structure of radicals in liquids, and to determine accurate absolute rate constants of fast radical reactions which are not easily accessible by conventional means[2].

We report experiments using a type of muon spectroscopy based on the effect of avoided level crossings[3,4]. Figure 1 illustrates magnetic energy levels of a three-spin system (μ^+, e^-, and nucleus k, chosen here to be a proton) at high magnetic fields B. Over most of the field range the energy eigenstates are stationary Zeeman states (denoted by α or β according to the orientation of the spins to the field). Crossing of states may be avoided by Fermi contact or dipolar interaction of the spins. In these regions, eigenstates are oscillating mixtures of pure Zeeman states. Therefore, an oscillatory inversion of the muon spin along the field results whenever the interaction involves one state with muon spin α_μ and one with β_μ. The inversion is seen experimentally by monitoring changes in the muon spin orientation when a magnetic field longitudinal to the spin polarization of the muon beam impinging on the sample is scanned through the region of an avoided crossing. Resonances are observed as changes in decay positron count rates in detectors placed in the 'forward' and 'backward' directions relative to the initial spin polarization. The muon polarization is then proportional to the experimental asymmetry $\mathcal{A}$, where

$$\mathcal{A} = \frac{\sum N_f - \sum N_b}{\sum N_f + \sum N_b} \tag{1}$$

and $\sum N_f$ and $\sum N_b$ are the total number of positrons detected in the forward and backward directions respectively. Signals appear as dips in $\mathcal{A}$ as a function of field because the resonant process transfers muon polarization from the forward to the backward direction, enhancing $\sum N_b$ at the expense of $\sum N_f$.

Prominent resonances correspond to the magnetic selection rule $\Delta M = 0$, where $M = m_e + m_\mu + m_k$ is the total spin projection, and represent the exchange of spin polarization between the muon and a magnetic nucleus. To first order they occur at fields

$$B = \left| \frac{A_\mu - A_k}{2(\gamma_\mu - \gamma_k)} \right| \tag{2}$$

where A_i are the hyperfine couplings and γ_i the gyromagnetic ratios. Here, crossing of the levels is avoided only indirectly by coupling of both states to an energetically distant third state, but the isotropic Fermi contact interaction suffices to lift the degeneracy of energies[5]. A fundamentally different type of resonance with $\Delta M = 1$ occurs around

$$B = \left| \frac{A_\mu}{2\gamma_\mu} \right| \tag{3}$$

In contrast to the $\Delta M = 0$ resonance, the crossing of levels is

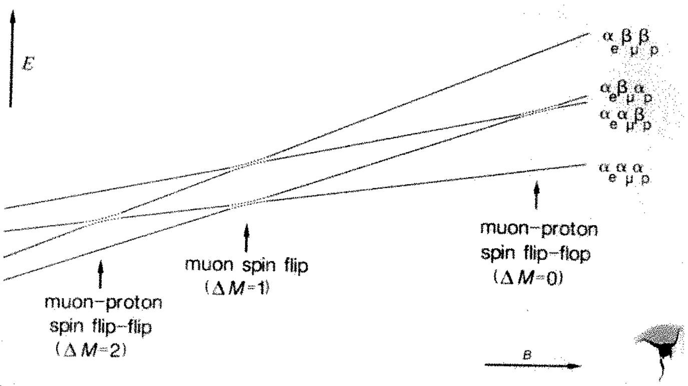
E
$\alpha_e \beta_\mu \beta_p$
$\alpha_e \beta_\mu \alpha_p$
$\alpha_e \alpha_\mu \beta_p$
$\alpha_e \alpha_\mu \alpha_p$
muon–proton
spin flip–flop
(ΔM=0)
muon spin flip
(ΔM=1)
muon–proton
spin flip–flip
(ΔM=2)
B

FIG. 1 High-field energy-level diagram for a three-spin system of an electron e, positive muon μ and proton p. States are labelled by the spin projections α or β along the field B. Avoided-level-crossing resonances occur when states with opposite muon spins become near-degenerate in energy, allowing the system to oscillate between them.

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avoided directly by coupling of two Zeeman states through the dipolar part of the hyperfine interaction[4]. Phenomenologically, this resonance can be viewed as muon precession in the remnant transverse component of the local field when the external field cancels the longitudinal component, so under isotropic conditions the resonance is lost. This is the case in a liquid, for example, where a radical averages dynamically over all orientations with the critical time being set by the hyperfine anisotropy, typically 10–30 MHz. Where mobility is restricted, as in a solid, this transition is expected to be observable and we have found in bulk benzene that it sets in extremely strongly below the freezing point[6]. A third type of resonance with $\Delta M = 2$ only appears under special orientations of the hyperfine coupling tensors to the magnetic field, and will not be considered further here.

The muonated cyclohexadienyl radical adsorbed on silica has been observed using the transverse-field muon-spin-rotation technique[7]. However, as radical formation was slow there was a dephasing of the muons forming radicals owing to significant precession in the precursor state. Thus the signal amplitude was greatly reduced and it was not feasible to determine reliable line widths as a function of temperature. The avoided-level-crossing technique is not as sensitive to formation rate because there is no precession in longitudinal fields, so radicals may be detected under much more dilute conditions with correspondingly longer radical formation times. Furthermore, hyperfine couplings are usually obtained for all magnetic nuclei in the radical, whereas the transverse-field technique provides only the muon coupling and thus a less detailed characterization of the species.

Our experiments were done on a SiO_2 powder sample of Cab-O-Sil grade EH-5 with an average grain diameter of 7 nm and a surface area of 380 $m^2\,g^{-1}$ (Cabot Corp., technical report). The powder (6 g) was placed in a stainless-steel cell with a muon entrance window of 50-μm stainless foil. The cell was baked under vacuum for 48 h at ~390 K to remove adsorbed water, leaving ~4 hydroxyl groups nm^{-2} at the surface (Cabot Corp., technical report). Based on a molecular cross-section of 0.40 nm^2, 0.75 g of degassed benzene was condensed into the cell to form a monolayer coverage[8], and the vacuum connection then sealed. Measurements were made using muons produced at the accelerator of the Paul Scherrer Institute. The sample was placed in the centre of a magnet of variable longitudinal field and held at a controlled temperature during measurements. Counts of decay positrons were accumulated at each field value for a period of ~30 s and the asymmetry $\mathcal{A}$ calculated as above.

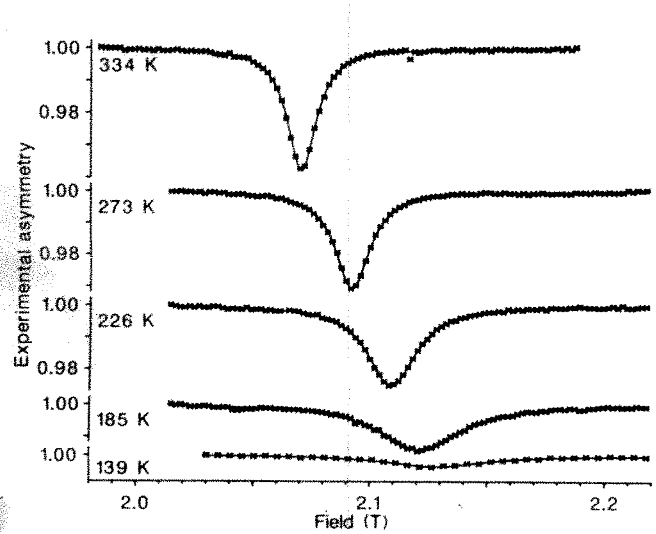

FIG. 2 $\Delta M = 0$ avoided-level-crossing resonances obtained from the methylene proton in muonated cyclohexadienyl radicals in a monolayer of benzene on 7-nm SiO_2 grains. The data were fitted to a lorentzian resonance on a cubic background, which has been removed in these displays; the solid curves give the fitted resonances.

At 334 K we observed a strong avoided-level-crossing resonance at 2.070 T and two weaker ones at 2.885 T and 2.945 T. They are all of the $\Delta M = 0$ type, involving different protons, and identify the muonated cyclohexadienyl radical which in the liquid has $A_\mu = 513$ MHz and $A_k = 126$ MHz (methylene proton), −25 MHz (ortho protons), +7.5 MHz (meta protons) and −36 MHz (para proton)[9]. Equation (2) thus associates the resonances with the methylene, ortho and para protons respectively. The methylene proton signal was studied at 12 temperatures in the range 139–334 K. Figure 2 shows representative signals where a slowly varying background due to changes in beam optics with magnetic field has been subtracted from the data. The change in resonance position is a reflection of the temperature dependence of the hyperfine couplings. The line shape is lorentzian at all temperatures, signifying isotropic conditions. We note that the $\Delta M = 1$ resonance around 1.9 T, which is a good indicator of anisotropy, was not detected at any temperature, so we can immediately conclude that there is no residual anisotropy in the C_6H_6Mu/Cab-O-Sil system over the temperature range studied.

Comparison of the signal amplitude with that observed in bulk liquid benzene reveals that about two-thirds of the muons reach a C_6H_6Mu radical state in the Cab-O-Sil sample at 334 K. The yield decreases continuously with temperature until the signal disappears below 139 K. We ascribe this to the slower thermal diffusion of the muons out of the silica grains and a lower radical formation rate at the lower temperatures.

The heat of adsorption of benzene onto a fully hydroxylated silica surface is 45 kJ mol^{-1}, attributed to polarization of the benzene π-orbitals by the hydroxyl groups[10]. In the absence of any evidence to the contrary, it is reasonable to assume that the same binding mechanism is true for the radical. The avoided-level-crossing resonance is shifted to higher fields, relative to the liquid, by about 5 mT at comparable temperatures, corresponding to an increase in the hyperfine coupling constants of the two methylene nuclei (muon and proton) of ~0.4%. An increase in the muon coupling was also seen in the transverse-field experiment[7], so the environment of the radical is evidently different from that in the liquid. A similar effect has been observed by electron spin resonance for the methyl radical adsorbed on vycor in comparison to *n*-hexane solution[11].

The line width is 7.6 mT HWHM (half width at half maximum) at the highest temperature. This exceeds the intrinsic width of 6.04 mT by only 25% and is close to the value observed in liquid benzene, illustrating similar mobilities for the radicals in the two environments at this temperature. The width increases continuously with decreasing temperature, in total by a factor of five over the whole range, indicating that the fluctuation of the hyperfine interaction becomes slower due to decreasing mobility of the radical. Three types of motion could be responsible for line-width effects. The first is rotation about an axis perpendicular to the surface, leading to a cylindrical Hamiltonian by partial averaging of the hyperfine anisotropy. It has been demonstrated for such a case in a plastic crystal that the $\Delta M = 1$ resonance is very strong even for small anisotropies[12]. The absence of this resonance at all temperatures means that the motion is not simply uniaxial. The second type of motion is rotation about an axis parallel to the surface. For a planar species like C_6H_6Mu this is more likely a flip, that is a jump between two orientations with identical hyperfine interactions, which also would not lead to the disappearance of the $\Delta M = 1$ resonance. The third motion to be considered is translation. On a flat surface this would not affect the orientation of the radical with respect to the magnetic field, but for a more or less spherical grain with a non-uniform surface it would very quickly produce an average over all orientations and hence the loss of the $\Delta M = 1$ signal.

Translation seems to be the only type of motion that can explain our results. Clearly, the $\Delta M = 0$ resonance shows linewidth effects because it has a different dependence on hyperfine couplings and senses motion on a different timescale to the $\Delta M = 1$ transition. Models for quantitative analysis of line broadening exist so far only for chemical reactions and spin exchange effects[13] but will have to be developed for reorientational motion. Qualitatively, our present observations show that despite a reduction in mobility at low temperatures, complete randomization of orientation of cyclohexadienyl radicals on a silica surface is still achieved in $\leqslant 10$ ns. Furthermore, it is interesting to note that over the temperature range studied there is no evidence of a phase transition in the adsorbed benzene film such as, for example, two-dimensional melting which has been observed for a benzene monolayer on graphite at ~ 130 K (ref. 14).

The main advantage of muon spectroscopy for studying surface-adsorbed radicals lies in the sensitivity of the technique. The high degree of spin polarization ($\sim 100\%$) and the single-particle detection mode allow it to work with concentrations down to just one muonated radical at a time in the entire sample. Electron-spin-resonance detection, for comparison, needs $\sim 10^{12}$ radicals in the cavity, corresponding to a concentration which usually cannot be maintained at temperatures where the radicals become mobile, as high mobility allows the radicals to diffuse together and terminate by combination (for example, to dicyclohexadiene in this case) or disproportionation (for example, to benzene and cyclohexadiene). The non-transparent and inhomogeneous nature of most heterogeneous catalysts on the other hand does not permit the use of optical methods. The avoided-level-crossing technique presented here seems to be suitable to fill the gap, and it is expected that it will allow the investigation of structure and dynamic behaviour of radicals on further materials of catalytic interest, such as zeolites and metal-loaded oxides, and that theory will be developed to permit the extraction of correlation times and activation energies from experimental data. □

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1. Schenck, A. *Muon Spin Rotation Spectroscopy. Principles and Applications in Solid State Physics.* (Adam Hilger, Bristol, 1985).
2. Roduner, E. *The Positive Muon as a Probe in Free Radical Chemistry* Lecture Notes in Chemistry 49 (Springer, Berlin, 1988).
3. Abragam, A. *C. r. hebd. Séanc. Acad. Sci., Paris II* **299**, 95–99 (1984).
4. Kiefl, R. F. *Hyperfine Interact.* **32**, 707–720 (1986).
5. Heming, M. *et al. Chem. Phys. Lett.* **128**, 100–106 (1986).
6. Roduner, E. *Chimia* **43**, 86–97 (1989).
7. Heming, M. *Z. Phys. Chem. Neue Folge* **151**, 35–50 (1987).
8. Gregg, S. J. & Sing, K. S. W. *Adsorption, Surface Area and Porosity* (Academic, London, 1967).
9. Percival, P. W. *et al. Chem. Phys. Lett.* **133**, 465–470 (1987).
10. Kiselev, A. V. & Lygin, V. I. *Infrared Spectra of Surface Compounds* (Wiley, New York, 1975).
11. Fischer, H. & Hefter, H. *Z. Naturf.* **23a**, 1763–1765 (1968).
12. Roduner, E., Reid, I. D., Riccò, M. & De Renzi, R. *Ber. Bunsenges. phys. Chem.* **93**, 1194–1197 (1989).
13. Heming, M. *et al. Chem. Phys.* **129**, 335–350 (1989).
14. Boddenberg, B. & Grosse, R. *Z. Naturf.* **42a**, 272–274 (1987).

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Efficient photoelectrochemical solar cells from electrolyte modification

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PHOTOELECTROCHEMICAL solar cells (PECs)[1–3] have shown energy conversion efficiencies approaching 13% in sunlight[4], and up to 15% in simulated insolation[5]. Of these, only those incorporating n-cadmium chalcogenide electrodes have been demonstrated to be conducive to thin film[6] or *in situ* storage systems[7]. Previous studies of photoelectrochemical current and voltage limitation[2,3,5,8,9] have focused on modification of the semiconductor electrode. Here we take the alternative approach by demonstrating that energy conversion can be improved by prevention of electrode surface modification and by systematic modification of the electrolyte. Electrolyte modification entails investigations of the primary photo-oxidized species, the nature of the counter ion, the distribution of species in solution, and related competing reactions. Optimization of the distribution of species and addition of cyanide to n-CdSe/([KFe(CN)$_6$]$^{2-/3-}$)$_{aq}$ PECs enhances the available voltage and the ease of charge transfer, and suppresses related decomposition products. The resultant PEC achieves an open-circuit potential of 1.2 V, an efficiency of 16.4%—the highest for any wide-band-gap (1.7 eV) solar cell (solid state or photoelectrochemical)—and a 100-fold improvement in photocurrent lifetime. Each of these represents a step towards realization of a viable PEC.

The highest conversion efficiencies in n-CdSe/[Fe(CN)$_6^{3-/4-}$]$_{aq}$ solar cells, 12 to 14%, have been reported using electrolytes containing a 1:1 ratio of Fe(CN)$_6^{4-}$ to Fe(CN)$_6^{3-}$ salts in a highly alkaline environment[8,10]. The observed photocurrents are, however, highly unstable and decay within hours[6]. Attempts to enhance the photoconversion stability of these systems have included cationic surface modification of the n-CdSe photoelectrode[9] and isolation of the wavelength dependence of the surface instability[11].

Photo-oxidative processes in n-CdSe/Fe(CN)$_6^{3-/4-}$ PECs are typically represented as

$$\mathrm{Fe(CN)_6^{4-}} + (h\nu) \rightarrow \mathrm{Fe(CN)_6^{3-}} + \mathrm{e^-} \qquad (1)$$

The role of the various redox-active constituents in constraining n-CdSe/Fe(CN)$_6^{3-/4-}$ photo-oxidative energy conversion has not been previously investigated, however, and ferricyanide and ferrocyanide salts show complex aqueous equilibria. In a series of studies on n-cadmium chalcogenide aqueous polysulphide PECs, we have emphasized the importance of isolating the active electrolytic constituents and identifying the redox reactions pertinent to overall energy conversion[12]. This approach is expanded in this study of n-CdSe ferri/ferrocyanide PECs. Monovalent cation salts of ferri and ferrocyanide, M$_3$Fe(CN)$_6$ and M$_4$Fe(CN)$_6$ can include the 22 aqueous equilibria relations:

$$\mathrm{H}_x\mathrm{M}_y\mathrm{Fe(CN)}_6^{4-x-y} + \mathrm{H}^+ \rightleftarrows \mathrm{H}_{x+1}\mathrm{M}_y\mathrm{Fe(CN)}_6^{3-x-y}$$
$$(x = 0 \text{ to } 3;\ y = 0 \text{ to } 3-x)$$
$$\mathrm{H}_x\mathrm{M}_y\mathrm{Fe(CN)}_6^{4-x-y} + \mathrm{M}^+ \rightleftarrows \mathrm{H}_x\mathrm{M}_{y+1}\mathrm{Fe(CN)}_6^{3-x-y}$$
$$(y = 0 \text{ to } 3;\ x = 0 \text{ to } 3-y) \qquad (2\text{–}14)$$
$$\mathrm{H}_x\mathrm{M}_y\mathrm{Fe(CN)}_6^{3-x-y} + \mathrm{H}^+ \rightleftarrows \mathrm{H}_{x+1}\mathrm{M}_y\mathrm{Fe(CN)}_6^{2-x-y}$$
$$(x = 0 \text{ to } 2;\ y = 0 \text{ to } 2-x)$$
$$\mathrm{H}_x\mathrm{M}_y\mathrm{Fe(CN)}_6^{3-x-y} + \mathrm{M}^+ \rightleftarrows \mathrm{H}_x\mathrm{M}_{y+1}\mathrm{Fe(CN)}_6^{2-x-y}$$
$$(y = 0 \text{ to } 2;\ x = 0 \text{ to } 2-y) \qquad (15\text{–}23)$$

Thermodynamic constraints for some of these equilibria have been investigated[13–15]. Our numerical iterative analysis, applying these known equilibria for M = K in the high-pH electrolytes we use here, shows that the dominant species in solution are the monocationic species KFe(CN)$_6^{3-}$ and KFe(CN)$_6^{2-}$, and the photoinduced oxidative process may be represented

$$\mathrm{KFe(CN)_6^{3-}} + (h\nu) \rightarrow \mathrm{KFe(CN)_6^{2-}} + \mathrm{e^-} \qquad (24)$$

Equation (24) is consistent with the findings[16] that electrochemical ferrocyanide oxidation (in the dark) is first order with respect to cation, and that potassium and caesium salts are electrocatalytically superior to the other alkali ferrocyanides. Here we have used the potassium salts.

An improvement in measured photocurrent stability with increase in pH is seen in comparing pH 3.8 and pH 13.3 electrolytes (Fig. 1, curves a, b). Photocurrent stability of about one hour is observed in the high-pH electrolyte (Fig. 1, curve b), comparable to the best previous n-CdSe/Fe$(CN)_6^{3-/4-}$ PEC stability[9]. Our measurements at high pH and at constant illumination, for a wide variation of $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ concentrations in n-CdSe/Fe$(CN)_6^{3-/4-}$ resulted in PECs that yield similar (to within ±2%) short-circuit photocurrents, but widely varying stability. Methods to measure light-pulsed transient photocurrents have been developed to probe PEC kinetics[17] but the slow (ms) response of that methodology provides only a qualitative measure of interfacial kinetics[18]. Here we have measured, under constant photon flux, potential-stepped transient photocurrent (PSTP) time response as a probe of PEC kinetics. PSTP is comparable to or faster than the interfacial double-layer charging time, and is only limited by electronic time constraints (20 μs) of the potentiostat. We compared PSTP time response of an n-CdSe electrode immersed in aqueous electrolytes containing constant concentration of $K_4Fe(CN)_6$ as the $K_3Fe(CN)_6$ concentration was varied by three orders of magnitude. It is evident that charge transfer is most facile at a ratio of ~20:1 between $KFe(CN)_6^{3-}$ to $KFe(CN)_6^{2-}$ salts (Fig. 2). We found that this optimal ratio is not sensitive to changes in pH, total salt or $K_4Fe(CN)_6$ concentrations.

The transient photocurrent measurements indicate that at a $KFe(CN)_6^{3-}$: $KFe(CN)_6^{2-}$ concentration ratio of ~20:1, more photogenerated carriers should participate in the constructive solution redox process (equation (24)) with fewer photogenerated carriers participating in photocorrosive processes. We do observe a substantial enhancement in photocurrent stability for the n-CdSe/KFe$(CN)_6^{2-/3-}$ system for this concentration ratio (Fig. 1, curves b, c); other electrolytes resulted in decreased photocurrent stability.

In addition to equations (2-23), a variety of other redox and substitution reactions contribute to the aqueous ferri/ferrocyanide system. In saturated phase and low-pH systems, the relatively insoluble cyanic acids and Prussian blue salts dominate[19]. Increase of solution pH substantially enhances n-CdSe/KFe$(CN)_6^{2-/3-}$ PEC conversion efficiences and cell stability. Various related properties were investigated, but the basis of this pH effect was not understood[10]. We propose that this suppression of photocorrosion and increase in stability are secondary effects that are the result of photoinduced electrolyte (as opposed to electrode) instability. Electrolyte photodecomposition occurs according to[20]

$$\begin{aligned} Fe(CN)_6^{4-} + H_2O &\rightleftarrows Fe(CN)_5H_2O^{3-} + CN^- \\ CN^- + H_2O &\rightleftarrows HCN + OH^- \end{aligned} \qquad (25)$$

The energy of activation of electrolyte photodecomposition is 19.8 kcal mol^{-1}; the rate is maximum at pH ~4 and falls off rapidly with increasing pH[20]. $Fe(CN)_5H_2O^{3-}$ has been shown to be highly reactive[21]. On illumination of the n-CdSe/KFe$(CN)_6^{2-/3-}$ PECs, we detect spectroscopically significant consumption of $KFe(CN)_6^{3-}$ and significant cyanide generation in solutions at pH 3.8 (adjusted by HCl addition), and none in solutions at pH > 13 (adjusted by addition of KOH). This is consistent with the poor stability and rapid photoinduced solution decomposition in the low-pH electrolyte (Fig. 1, curves a, b). When we use the pH 3.8 solution as a photoelectrolyte for the PEC, a brown photoelectrode overlayer forms within seconds, consistent with known $KCdFe(CN)_6$ and $Cd_4[KFe(CN)_6]_3$ salts[22,23]. The observed rate of overlayer formation diminishes by several orders of magnitude in the high-pH 0.25 m $K_4Fe(CN)_6$/0.0125 m $K_3Fe(CN)_6$ electrolyte. Consumption of $Fe(CN)_6^{4-}$, equation (25), will diminish availability of the (photo)active hexacyanide species leading to reduced PEC performance, equation (24). The effect of photoinduced decomposition of electrolyte on the long-term stability of n-CdSe/Fe$(CN)_6^{2-/3-}$ photoelectrochemical cells is readily detected (Fig. 1, curves c, d). Further improvement in photocurrent stability occurs when electrolyte is renewed by continuously removing photoinduced electrolyte decomposition products from the cell (Fig. 1 inset, curve d).

Consistent with the stability measurements (Fig. 1, curves c, d), added cyanide may be expected to slow down the electrolyte decomposition by enhancing the reverse reaction, equation (25). The presence of CN_{aq}^- also affects solubility. Addition of Cd_{aq}^{2+} (as dissolved $CdCl_2$) to alkaline $K_3Fe(CN)_6/K_4Fe(CN)_6$ electrolytes immediately precipitates insoluble cadmium ferri/ferrocyanide salts. But when 0.1 m KCN is incorporated in the same electrolytes added Cd_{aq}^{2+} is entirely soluble up to a $CdCl_2$:KCN concentration ratio of 1:4, dissolving as the complex $Cd(CN)_{4aq}^{2-}$. Crystalline CdSe remains insoluble in either electrolyte. Cyanide can therefore remove otherwise insoluble alkali cadmium ferri/ferrocyanide photodecomposition products[9], preventing surface passivation and maintaining an active clean CdSe surface morphology.

Further dramatic improvements in photocurrent stability are

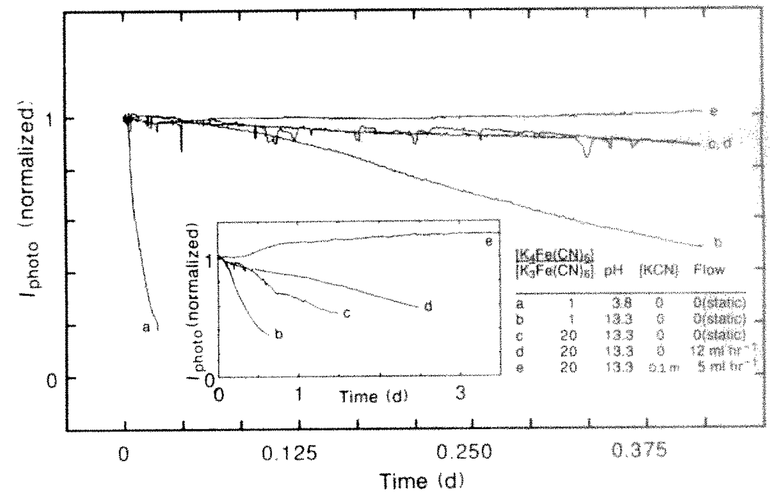

FIG. 1 Photocurrent stability of single-crystal n-CdSe immersed in a variety of 0.25 m $K_4Fe(CN)_6$ solutions. Inset: Long-term stability. 0.200-cm^2, 1-ohm-cm n-CdSe (Cleveland Crystal) was mounted with the (1120) plane exposed, and was pretreated with successive 5-μm, 1-μm, 0.35-μm and 0.05-μm mechanical polishing, chemically etched in 2% bromine/methanol (by volume) and photoetched[4] in 1.0 m Na_2SO_4. Electrolytes were freshly prepared with analytical grade reagents (Aldrich Chemicals) in deionized distilled water. Before use all electrolytes were purged with high-purity argon. Intensity of the 100-W tungsten-halogen illumination was initially varied by distance to provide an illumination of ~25 mW cm^{-2} for data presented in curves a to d and ~40 mW cm^{-2} for data presented in curve e, calibrated by outdoor comparison to air mass 1.5 insolation. Photocurrents measured in curves a–e were normalized relative to the initial photocurrents (initial photocurrents of 2.10, 2.97, 2.79, 3.12 and 6.77 mA cm^{-2} were measured for data presented in curves a–e). Photocurrents reported in curve e increased to 8.05 mA cm^{-2}. Potential was applied by a PINE AFRDE4X1 potentiostat in a conventional three-electrode configuration (Pt reference and 6-cm^2 Pt-mesh counter electrodes). In the flow-through study, we compared photocurrent stability with and without electrolyte renewal. Electrolyte was delivered into the cell without turbulence by gravity feed. At higher light intensities (90 mW cm^{-2}), in a PEC with an alkaline electrolyte containing optimized $K_3Fe(CN)_6/K_4Fe(CN)_6$ concentrations with added cyanide (as in curve e), the photocurrent remained stable for >5,000 C cm^{-2} in the flow cell and for >1,000 C cm^{-2} without electrolyte renewal.

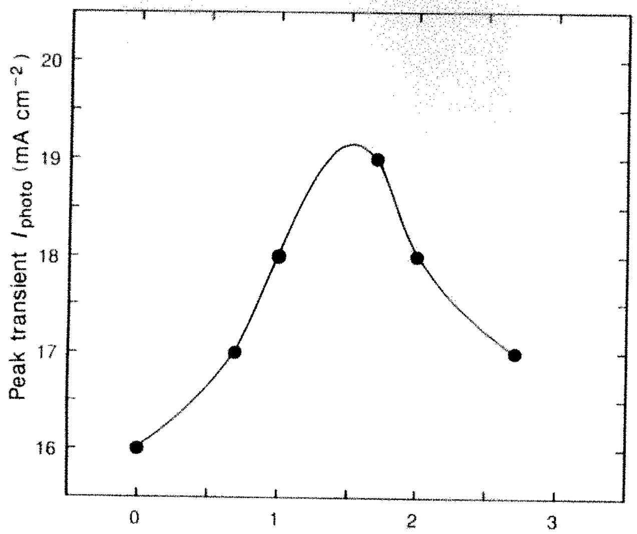

FIG. 2 Peak transient photocurrent in n-CdSe/[Fe(CN)$_6^{3-/4-}$]$_{aq}$ cells measured over a wide range of $K_3Fe(CN)_6$ concentrations in 0.5 m $K_4Fe(CN)_6$ and 1.0 m KOH. The PEC was initially maintained by potentiostat at the open-circuit voltage. Peak photocurrent response was measured under a constant illumination of 25 mW cm^{-2}, after the application of a 300-mV anodic step. Potential steps were applied by a Heath 1274 sweep-function generator (200-ns rise time) to an AFRDE4X1 potentiostat (20-μs rise time) in a conventional three-electrode configuration (Pt reference and 10-cm^2 Pt-mesh counter electrodes). Current output was digitized (2 μs per data pt, 16 K data per scan) by an 80286-interfaced Heath SD-5000 Digital Scope. Electrode preparation, three-electrode cell configuration and illumination source are described in Fig. 1.

therefore expected and observed on addition of cyanide (Fig. 1, curve e). At more-intense 90 mW cm^{-2} illumination, the cell remains stable for the photocurrent generation of over 5,000 C cm^{-2}. The polished CdSe surface can lose over 20% reflectivity[24]. Our observed increase in photocurrent during the initial photoconversion (Fig. 1 inset, curve e) parallels an observed drop in CdSe surface reflectivity. We relate this to an initial dissolution and morphological improvement of the surface, as indicated by Cd^{2+} atomic absorption spectrophotometry (Perkin Elmer 370) of the static PEC electrolyte, coulombic count and a photoelectrode mass loss of ~ 0.03 g cm^{-2} (equivalent to ~ 30 C cm^{-2}). Following this initial dissolution, photocorrosion comprises $\leq 1\%$ of total photocurrent, as determined by subsequent atomic absorption measurements, coulombic count and photoelectrode mass loss. Cyanide prevents passivation and chemical deposition at the CdSe surfaces, maintaining a clean surface morphology for facile charge transfer. This is is contrast with the varying degrees of success in previous attempts to modify photoelectrode surfaces (including metal[2], polymer[3], chalcogenide[8], electrocatalyst[5] and mixed alkali salt[9] surface modifiers). Thin-film n-CdSe photoelectrodes are expected to be several orders of magnitude more stable than comparable single-crystal n-CdSe PECs[6].

There is a third beneficial effect of CN^-_{aq} addition. n-CdSe/KFe(CN)$_6^{2-/3-}$ PECs containing 0.1 m KCN show open-circuit photovoltages 250 mV more negative compared with electrolytes without cyanide, and E_{redox} {KFe(CN)$_6^{2-/3-}$} is not shifted (Fig. 3). Enhancement of the photovoltage is accompanied by a decrease in dark current (Fig. 3). The shift in measured open-circuit voltage (V_{oc}), $\sim$0.12 V per decade [CN^-_{aq}] and $\sim$0.03 V per decade [$Cd(CN)^{2-}_{4aq}$] (Fig. 3 inset), is consistent with a decomposition reaction

$$CdSe + 4CN^- \rightarrow Cd(CN)_4^{2-} + Se + 2e^- \qquad (26)$$

As discussed, little CdSe decomposition is observed with passage of $>5,000$ C cm^{-2}. It is interesting that in accordance with equation (26), there is a thermodynamic shift of photo-induced oxidation equation (24), without the faradaic losses suggested by equation (26). Electrochemical (dark) oxidation of CN^- on Pt electrodes is insignificant at these potentials and concentrations of KCN. An analysis of the adsorption/flat-band potential shift, consistent with our model for ion/semiconductor interactions[25], is in progress. n-CdSe chalcogenide photovoltages of 1.0 V have been previously observed in dithiocarbamate solutions and attributed to strong specific adsorption[26]. Photovoltages in n-GaAs PECs in excess of those predicted for analogous solid-state junctions have also been observed[27].

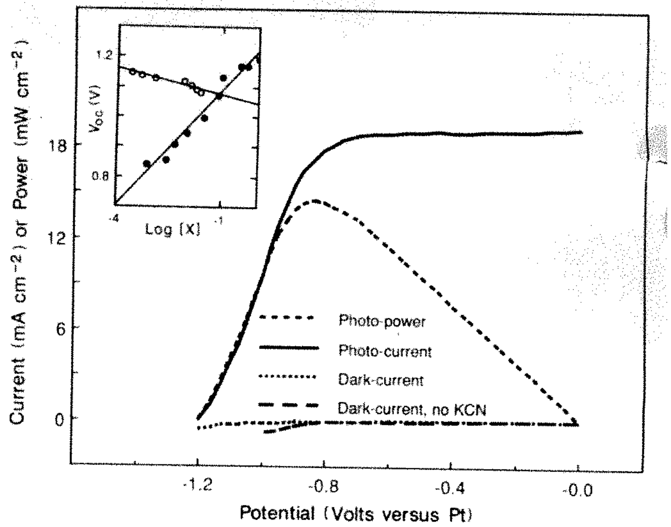

FIG. 3 Outdoor potentiostatic characteristics for an illuminated (88 mW cm^{-2} insolation) or dark n-CdSe/0.25 m $K_4Fe(CN)_6$, 0.0125 m $K_3Fe(CN)_6$, 0.1 m KCN, 0.5 m KOH PEC. Electrode preparation and three-electrode cell configuration are described in Fig. 1. The results were reproduced with several n-CdSe electrodes of area 0.05–0.20 cm^2, at potential sweep rates of 0.5–2 mV s^{-1}. Measured solar-to-electrical conversion efficiency varied from 15.6 to 16.4%. Insolation was calibrated using an Eppley 8-48 radiometer. 'no KCN' indicates an electrolyte 0.25 m $K_4Fe(CN)_6$, 0.0125 m $K_3Fe(CN)_6$ and 0.5 m KOH. On Pt, the 0.25 m $K_4Fe(CN)_6$, 0.0125 m $K_3Fe(CN)_6$ and 0.5 m KOH electrolytes with and without 0.1 m KCN, had respective potentials of 0.132 and 0.133 V relative to the AgCl reference electrode. Inset: The variation of open-circuit photovoltage (under 80 mW cm^{-2} tungsten-halogen illumination) in n-CdSe/0.25 m $K_4Fe(CN)_6$, 0.0125 m $K_3Fe(CN)_6$, 0.5 m KOH PECs containing varying concentrations of CN^- (filled circles), added as KCN (no added $Cd(CN)_4^{2-}$), and in constant 0.1 m KCN with varying concentrations of $Cd(CN)_4^{2-}$ (open circles), added as 4:1 [KCN]:[$CdCl_2$].

Outdoors, the n-CdSe/KFe(CN)$_6^{2-/3-}$ solar cell with an alkaline electrolyte containing optimized $K_3Fe(CN)_6/K_4Fe(CN)_6$ electrolyte and added KCN shows a stable conversion efficiency $>16\%$ (fill factor of 0.63) and an open-circuit photovoltage of 1.20 V (Fig. 3). The quantum yield of the alkaline n-CdSe/Fe(CN)$_6^{3-/4-}$ PEC is known to approach unity[10]. CdSe with a 1.7-eV band gap has a limiting current density of 18.45 mA cm^{-2} at air mass 1.5 (84.4 mW cm^{-2}) insolation[28]. Our measured photocurrents, normalized to air mass 1.5, range from 17.3 to 18.2 mA cm^{-2} and are consistent with small reflectance or other losses.

In a future paper we will summarize the numerical calculations of the solution equilibria, and the effects of the variation of cation and ligand $M_xFe(CN)_5L$ (for L = CN, NO, NO_2, NH_3 and $Fe(CN)_5$, and for M = NH_3, Ca, Li, Na, K and Cs).

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1. Parkinson, B. *Accounts Chem. Res.* **17,** 431–437 (1984).
2. Miller, B., Heller, A., Menezes, S. & Lewerenz, H. J. *Discuss. Faraday Soc.* **70,** 223–232 (1981).
3. Noufi, R. *Conf. Rec. IEEE Photovoltaic Spec. Conf. 16th,* 1293–1298 (1982).
4. Licht, S. *et al. Appl. Phys. Lett.* **46,** 608–610 (1985).
5. Tufts, B. J. *et al. Nature* **326,** 861–862 (1987).
6. Licht, S. *J. phys. Chem.* **90,** 1096–1099 (1986).
7. Licht, S., Hodes, G., Tenne, R. & Manassen, J. *Nature* **326,** 863–864 (1987).

8. Reichman, J. & Russak, M. A. *J. electrochem. Soc.* **131,** 796–798 (1984).
9. Rubin, H. D., Humphrey, B. D. & Bocarsley, A. B. *Nature* **308,** 339–341 (1984).
10. Freeze, K. W. Jr. *Appl. Phys. Lett.* **40,** 275–277 (1982).
11. Rubin, H. D., Arent, D. J. & Bocarsley, A. B. *J. electrochem. Soc.* **132,** 523–524 (1985).
12. Licht, S. *Nature* **330,** 148–151 (1987).
13. Jordan, J. & Ewing, G. J. *Inorg. Chem.* **1,** 587–591 (1962).
14. Hanania, G. I. H., Irvine, D. H., Easton, W. A. & George, P. *J. phys. Chem.* **71,** 2022–2030 (1967).
15. Capone, S., de Robertis, A., de Stafano, C. & Scarella, R. *J. chem. Res.* **1,** 412–413 (1986).
16. Peter, L. M., Durr, W., Bindra, P. & Gerischer, H. *J. electroanalyt. Chem.* **132,** 31–50 (1976).
17. Lando, D., Manassen, J., Hodes, G. & Cahen, D. *J. Am. chem. Soc.* **101,** 3969–3971 (1979).
18. Licht, S., Tenne, R., Flaisher, H. & Manassen, J. *J. electrochem. Soc.* **133,** 52–59 (1985).
19. Itaya, K., Akahoshi, H. & Toshima, S. *J. electrochem. Soc.* **129,** 1498–1500 (1982).
20. Asperger, S., Murati, I. & Pavlovic, D. *J. chem. Soc.* 730–736 (1960).
21. Lal, B. B. *Ind. chem. Soc. J.* **16,** 321–323 (1939).
22. Ayers, J. B. & Waggoner, W. H. *J. inorg. nucl. Chem.* **33,** 721–733 (1971).
23. Reihlen, H. & Zimmermann, W. *Justus Liebigs Annln Chem.* **475,** 101–119 (1929).
24. Heller, A., Chang, K.-C. & Miller, B. *J. Am. chem. Soc.* **100,** 684–688 (1978).
25. Licht, S. & Marcu, V. *J. electroanalyt. Chem.* **210,** 197–204 (1986).
26. Thackeray, J. W., Natan, M. J., Ng, P. & Wrighton, M. S. *J. Am. chem. Soc.* **108,** 3570–3577 (1986).
27. Abrahams, I. L. *et al. New J. Chim.* **11,** 157–165 (1987).
28. Henry, C. H. *J. appl. Phys.* **51,** 4494–4500 (1980).

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Heterogeneous reactions on nitric acid trihydrate

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HETEROGENEOUS reactions on the surfaces of polar stratospheric cloud particles[1–4] are now known to have a crucial role in the springtime decrease of polar ozone[5,6] by converting inactive chlorine reservoir species, $ClONO_2$ and HCl, to photochemically active forms, Cl_2 and HOCl, thus preparing the stratosphere for ozone destruction on the return of sunlight in the spring. There is abundant recent theoretical[7–9] and indirect observational[10–16] evidence for two main types of polar stratospheric cloud (PSC). The predominant form, type I, which comprises HNO_3 and H_2O in the form of frozen nitric acid trihydrate (NAT), can condense at 195 K in the stratosphere[9]. This is 5–7 K warmer than the condensation temperature of type II PSCs, which are composed mainly of water ice. To understand the surface chemistry of PSCs and to test current hypotheses concerning polar ozone loss, laboratory measurements of several key kinetic parameters are needed. Here we report the first direct measurements of the reaction probabilities at stratospheric temperatures for two important heterogeneous reactions on NAT. Additionally, we report the sticking coefficients and solubilities of HCl and NAT, which are important in modelling physical-chemical processes in the stratosphere. Our results show that the conversion of the chlorine reservoir species can occur within a few days of the first appearance of type I PSCs during the polar winter.

The heterogeneously catalysed reactions of primary interest in the stratosphere are

$$ClONO_2 + HCl \rightarrow Cl_2 + HNO_3 \qquad (1)$$

$$ClONO_2 + H_2O \rightarrow HOCl + HNO_3 \qquad (2)$$

$$N_2O_5 + HCl \rightarrow ClNO_2 + HNO_3 \qquad (3)$$

$$N_2O_5 + H_2O \rightarrow 2HNO_3 \qquad (4)$$

Reaction (1) may be particularly important as it converts the chlorine in two inactive reservoir molecules into the volatile and photochemically active Cl_2 molecule. Reaction rates have been measured for reactions (1)–(4) on ice[17–21] but not on NAT.

Rates of reactions (1) and (2) were measured by using a fast-flow reactor coupled to a differentially pumped mass-spectrometer detection system (Fig. 1)[20,21]. Total pressures were ~0.4–2 torr at a temperature of 196 K; average flow velocities ranged from ~100 to 2,500 cm s^{-1}. Substrates were formed on the inner surface of the cooled reactor tube by vapour deposition at controlled rates near 0.5 g h^{-1}. Compositions were varied from 37–57 weight per cent (wt%) HNO_3 to cover the range of NAT compositions[9] (45–53 wt% HNO_3) believed to be stable in the stratosphere. As used here, the term NAT includes both H_2O-rich and HNO_3-rich ices near the composition of pure nitric acid trihydrate, 53.83 wt% HNO_3. The HNO_3-ice substrates seemed to be frost-like crystalline solids and served as approximations of actual PSC aerosols.

In separate experiments, we also investigated the chemical composition of NAT substrates by means of Fourier transform infrared spectroscopy. Thin (~1 μm) substrates were deposited very slowly on an AgCl window which was maintained at 188 K. The infrared spectra were collected while the vapour deposition took place or after annealing the substrates for about an hour. The characteristic infrared absorption spectrum of NAT was observed, and the spectra indicate that the NAT substrates may contain hydronium and nitrate ions.

To measure the rate of reaction (1), the substrate is first doped with HCl by vapour deposition and then $ClONO_2$ is continuously admitted to the reactor through the injector (see Fig. 1). Figure 2 shows the change in the reactor-exit concentrations of the $ClONO_2$ reactant and Cl_2, the product of reaction (1),

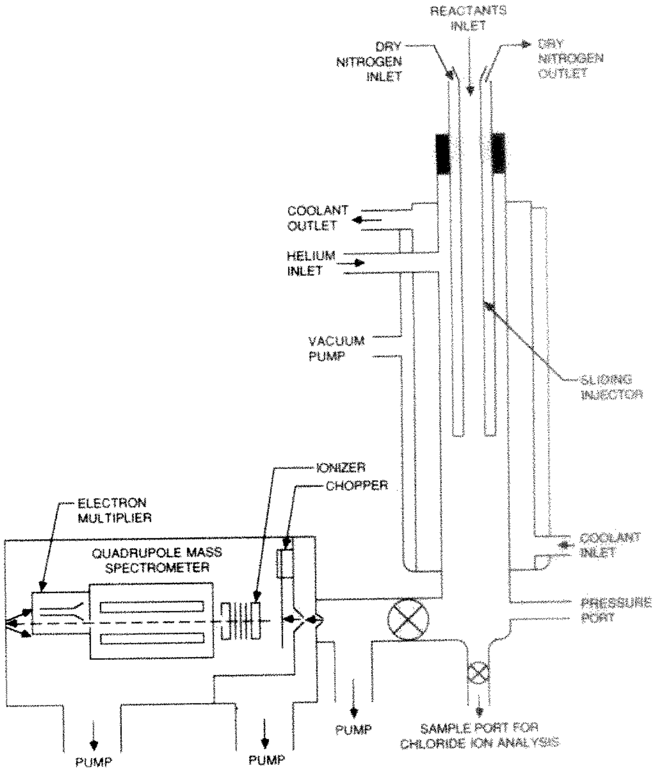

FIG. 1 Schematic diagram of fast-flow reactor system. A thin (~50 μm) NAT substrate is formed on the internal surface of the cylindrical reactor (1.76 cm in diameter) for a distance of 30 cm starting at the reactor exit by flowing He carrier gas saturated with HNO_3 and H_2O (at 293 K) through the injector after the reactor is cooled to operating temperature (196 ± 1 K). Usually, ~1 g of substrate is deposited. HCl is deposited in the substrate by flowing a mixture of He and HCl over the surface, in some cases saturating the substrate with HCl. Subsequently, He carrier gas at 0.4–2.0 torr is used to flow the reactants into the reactor. The HCl flows in through the main He inlet and the $ClONO_2$ flows in through the injector to prevent reaction except over the substrate. Following an experiment, the substrate is warmed and collected for analysis.

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as the contact time over the substrate is increased by moving the injector. The observed loss rate of $ClONO_2$ and the production rate of Cl_2 are used to obtain a first-order surface rate constant k_s by standard kinetics analysis techniques[20,21]. The parameter of interest in atmospheric modelling is the reaction probability γ which can be obtained from k_s by equating the reaction rate with the surface collision rate which depends on the total surface area; for a cylindrical reactor, this leads to the relation

$$\gamma = 2ak_s/(\omega + ak_s) \qquad (5)$$

where a is the reactor radius and ω is the molecular velocity of $ClONO_2$. Before using the above equation to calculate γ, it is necessary to correct the observed surface rate constants for the interaction of flow dynamics, diffusion and reaction[22]. Under our experimental conditions, corrections were less than 20% for $\gamma < 0.01$; but for $\gamma > 0.1$ the corrections were a factor of two or larger.

The observed reaction probabilities for $ClONO_2$ with HCl are shown in Fig. 3 as a function of the HCl concentration in the substrate. Values of γ range from 0.06 to 1.0 with an overall average of 0.3. Our observation of uniformly high reaction probabilities for all HCl concentrations used may indicate that the surface is saturated with HCl at the partial pressures, P(HCl), used in these experiments (0.1–0.3 mtorr). We observed no significant change in γ between successive runs on fresh substrate in which the P(HCl) was either held constant by a continuous flow of HCl into the reactor or allowed to drop to a much lower value (presumably its equilibrium level over the substrate). This may indicate that the surface remains saturated with HCl at these lower partial pressures or that the HCl is rapidly replenished at the surface from the interior. It is possible that at the much lower P(HCl)s typical of the stratosphere ($<10^{-6}$ torr) the NAT surfaces will not be saturated with HCl and γ will be correspondingly lower and a function of P(HCl). The observed reaction probabilities are independent of the HNO_3 substrate concentrations over the range studied (42–53.6 wt% HNO_3) on the water-rich side of pure NAT. At HNO_3 substrate concentrations greater than 54 wt%, HCl sorption decreases rapidly (see below). We were not able to maintain a constant HCl substrate concentration, and therefore could not carry out measurements of reaction (1) on acid-rich substrates. The good agreement between reaction probabilities determined from monitoring the $ClONO_2$ reactant concentration and these determined from monitoring the Cl_2 product concentrations (see Fig. 3) indicates that a true reaction probability was being measured, as opposed to the physical adsorption of $ClONO_2$. These observations are consistent with the results for water ice reported previously from our laboratory[20].

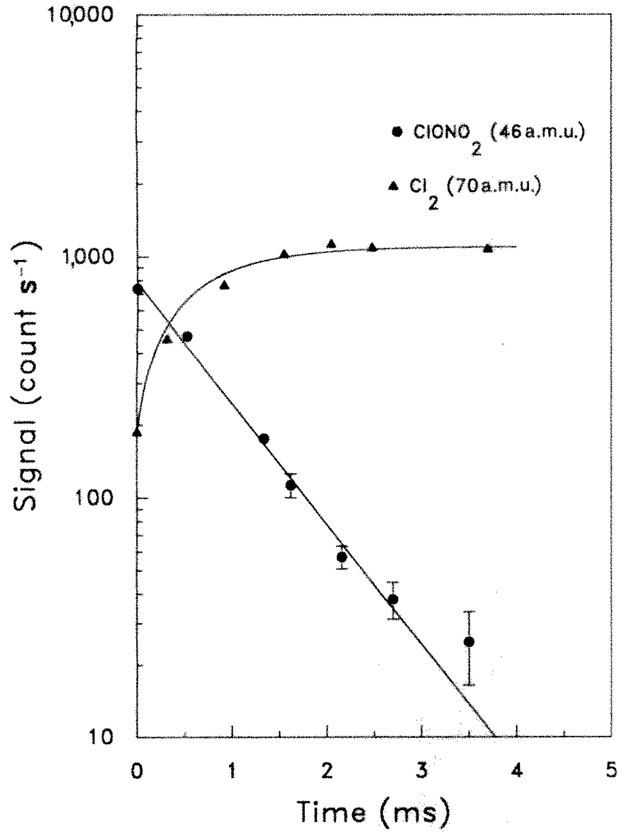

FIG. 2 Typical raw mass spectrometer signal, which is proportional to the reactor-exit concentration, for the $ClONO_2 + HCl \rightarrow Cl_2 + HNO_3$ reaction on a NAT substrate. The length of substrate exposed to $ClONO_2$ has been converted to time by dividing by the average flow velocity (1,856 cm s^{-1}). The NO_2^+ ion fragment from $ClONO_2$ is monitored with the mass spectrometer, and Cl_2 is monitored by its parent ion, Cl_2^+. The linear decay of the $ClONO_2$ signal on the logarithmic plot implies the reaction is first order with respect to $ClONO_2$; the slope of the line gives the first-order rate constant for reaction (1). The deviation of the $ClONO_2$ signal from linearity at long times is the result of interference from HNO_3 evaporating from the substrate; the HNO_3 produced in the reaction is incorporated in the substrate and not released to the gas phase immediately. The data are from successive runs on a substrate comprised of 53.2 wt% HNO_3 doped with 0.46 wt% HCl at a temperature of 196 K.

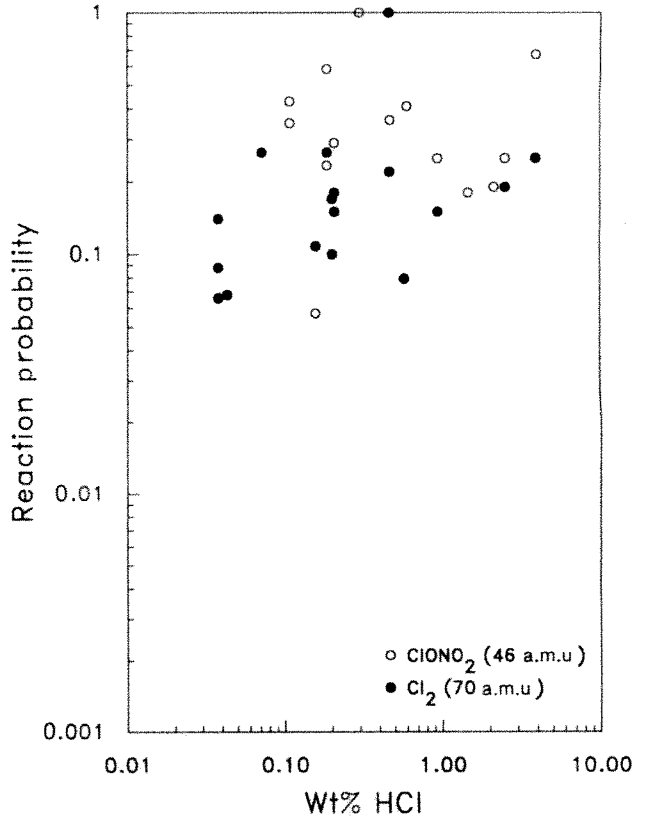

FIG. 3 The reaction probability γ of $ClONO_2$ with HCl on NAT substrate. The substrate HNO_3 composition varies from 42.0 wt% to 53.6 wt%. Reaction probabilities are obtained both from the decay of $ClONO_2$ and the growth of Cl_2. A correction for gas-phase diffusion in the reactor has been incorporated in the calculated reaction probabilities.

The γ of $ClONO_2$ on NAT in the absence of HCl was determined to be at least an order of magnitude smaller than that in the presence of HCl (0.02 ± 0.01 at 45–47 wt% HNO_3) and to fall sharply as the composition of pure NAT was approached. Measurements of the physical sticking coefficient of HCl on NAT substrates were carried out by using the same technique used to determine γ. The results showed similar

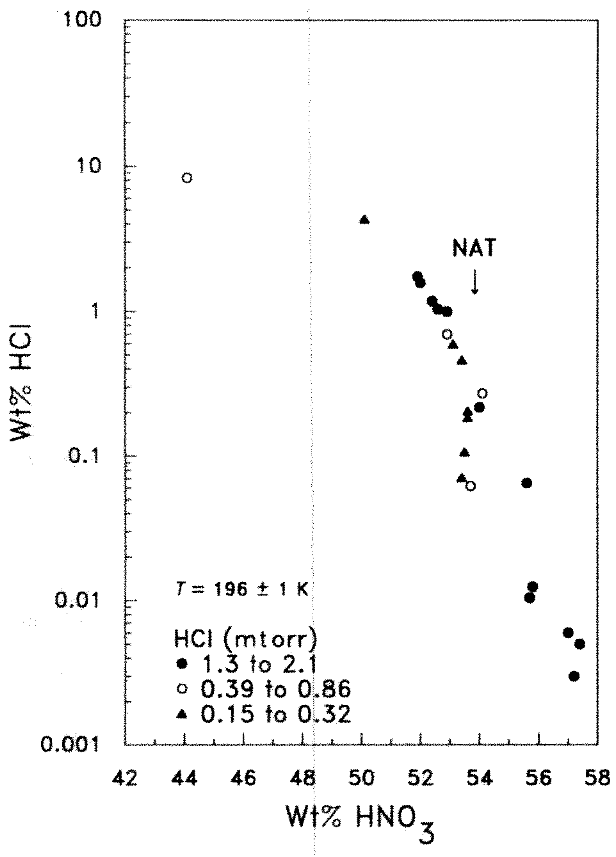

FIG. 4 Sorption of HCl by NAT as a function of wt% HNO_3. The arrow indicates the composition of pure NAT, 53.83 wt%. We calibrated the mass spectrometer with a given partial pressure of HCl (monitored with HCl^+) in the He flow. Equilibrium was assumed to occur when the signal for the HCl flowing over the substrate returned to the calibration level. The total acid content of the substrate is determined by standard acid–base titration, and the HCl content by means of a Cl^- selective electrode.

behaviour, falling from 0.05 ± 0.01 at 44–46 wt% HNO_3 to less than 10^{-4} at compositions greater than 55 wt% HNO_3.

The calculation of a reaction probability or a sticking coefficient requires a knowledge of the effective surface area of the substrate. The values reported above are based on the macroscopic external surface area of the substrate. Brunauer–Emmett–Teller (BET) measurements of the substrate's microscopic surface area give values ~10–100 times larger than the macroscopic area. The amount of this area that is available to the reacting species depends on the exact structure of the substrate. A model to take this effect into account is currently being developed. We estimate that reaction probabilities greater than 0.1 would be reduced by about a factor of 5, but measured probabilities less than 0.01 could be significantly reduced.

The substrates were also saturated with HCl to determine the equilibrium sorption of HCl by the NAT substrate. The results (Fig. 4) are in qualitative agreement with solubility measurements[23,24] made at much lower P(HCl)s (10^{-8}–10^{-5} torr), which required temperatures near the frost point (that is, excess water of hydration), before measurable amounts of HCl were taken up by NAT. We were not able to determine the mode of HCl incorporation in the NAT, and it may exist as a highly concentrated phase on the surface or at grain boundaries[25]. It is clear that more than simple physical adsorption is involved because a monolayer (based on a molecular area of 16×10^{-20} m^2 $molecule^{-1}$) of HCl condensed on a 3 m^2 g^{-1} solid gives ~0.1 wt% HCl—far less than our observation of at least 0.7 wt% HCl for substrates with less than 53 wt% HNO_3.

We may now consider the implications of our measurements for theories of the ozone-hole formation. For reaction (1), the present study gives a reaction probability, $\gamma \approx 0.1$–0.3, on the HCl-doped water-enriched type I ices which are expected under stratospheric conditions[15,23]; for reaction 2, $\gamma \approx 0.02$. Hence, HCl and $ClONO_2$ conversion to Cl_2 through reaction (1) can occur within one day and $ClONO_2$ activation through reaction (2) within one week[26]. The initial conversion of the chlorine reservoir thus occurs within a few days of the first appearance of type I PSCs in the polar winter season, and the chlorine reservoir remains activated as long as these nitric acid PSCs continue to form or NO_2 is absent (through denitrification). Our data suggest that, as type I PSCs are also common in the Arctic polar winter stratosphere[13,27,28], chlorine activation will also occur in the north.

The earlier work[23,24] and the present results show that type I cloud particles probably contain only ~0.5 wt% of HCl compared to ~50 wt% of HNO_3 whereas the ambient stratospheric ratio of $HCl:HNO_3$ is roughly 1:10. Thus, the chlorine reservoir cannot be significantly depleted by sedimentation of type I cloud particles (concurrent with denitrification).

The close coupling between type I PSCs and chlorine activation provides a mechanism for prolonging the ozone hole during the austral spring. The southern polar vortex is destabilized in October/November by warming of the vortex associated with the absorption of sunlight by ozone. If ozone is strongly depleted in October, the warming is reduced and the vortex remains cold and stable. PSCs are thus more persistent[29], chlorine continues to be activated[30], and the depleted levels of ozone can persist. The possibility of such a positive chemical/radiative/dynamical feedback mechanism is in part the result of heterogeneous chlorine activation on the particles of nitric acid ice, as defined by the present study.

Farman *et al.*[5] first attributed the development of the ozone hole to the accumulation of chlorofluorocarbons (CFCs) in the atmosphere. The chlorine released when CFCs are photolytically decomposed in the stratosphere can be activated on PSCs and deplete polar ozone in the spring[1-3]. Our results indicate that the observed onset of the ozone depletion is indeed connected to the buildup of CFCs, and that ozone depletion has probably never been strongly limited by chlorine activation on PSCs. □

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1. Solomon, S., Garcia, R. R., Rowland, F. S. & Wuebbles, D. J. *Nature* **321,** 755–758 (1986).
2. McElroy, M. B., Salawitch, R. J., Wofsy, S. C. & Logan, J. A. *Nature* **321,** 759–762 (1986).
3. Crutzen, P. J. & Arnold, F. *Nature* **324,** 651–655 (1986).
4. Hofmann, D. J., Harder, J. W., Rolf, S. R. & Rosen, J. M. *Nature* **326,** 59–62 (1987).
5. Farman, J. C., Gardiner, B. G. & Shanklin, J. D. *Nature* **315,** 207–210 (1985).
6. Stolarski, R. S. *et al. Nature* **322,** 808–811 (1986).
7. Toon, O. B., Hamill, P., Turco, R. P. & Pinto, J. *Geophys. Res. Lett.* **13,** 1284–1287 (1986).
8. McElroy, M. B., Salawitch, R. J. & Wofsy, S. C. *Geophys. Res. Lett.* **13,** 1296–1299 (1986).
9. Hanson, D. & Mauersberger, K. *Geophys. Res. Lett.* **15,** 855–858 (1988).
10. Austin, J., Remsberg, E. E., Jones, R. L. & Tuck, A. F. *Geophys. Res. Lett.* **13,** 1256–1259 (1986).
11. Poole, L. R. & McCormick, M. P. *Geophys. Res. Lett.* **15,** 21–23 (1988).
12. Hofmann, D. J., Rosen, J. M., Harder, J. W. & Hereford, J. V. *J. geophys. Res.* **94,** 11,253–11,269 (1989).
13. Rosen, J. M., Oltmans, S. J. & Evans, W. F. *Geophys. Res. Lett.* **16,** 791–794 (1989).
14. Pueschel, R. F. *et al. J. geophys. Res.* **94,** 11,271–11,284 (1989).
15. Fahey, D. W. *et al. J. geophys. Res.* **94,** 11,299–11,315 (1989).
16. Arnold, F., Schlager, H., Hoffmann, J., Metzinger, P. & Spreng, S. *Nature* **342,** 493–497 (1989).
17. Tolbert, M. A., Rossi, M. J., Malhotra, R. & Golden, D. M. *Science* **238,** 1258–1260 (1987).
18. Tolbert, M. A., Rossi, M. J. & Golden, D. M. *Science* **240,** 1018–1021 (1988).
19. Molina, M. J., Tso, T. L., Molina, L. T. & Wang, F. C-Y. *Science* **238,** 1253–1257 (1987).
20. Leu, M-T. *Geophys. Res. Lett.* **15,** 17–20 (1988).
21. Leu, M-T. *Geophys. Res. Lett.* **15,** 851–854 (1988).
22. Brown, R. L. *J. Res. natn. Bur. Stand.* **83,** 1–8 (1978).
23. Hanson, D. & Mauersberger, K. *Geophys. Res. Lett.* **15,** 1507–1510 (1988).
24. Hanson, D. & Mauersberger, K. *J. phys. Chem.* (in the press).
25. Wolff, E. W., Mulvaney, R. & Oates, K. *Geophys. Res. Lett.* **16,** 487–490 (1989).
26. Turco, R. P., Toon, O. B. & Hamill, P. *J. geophys. Res.* **94,** 16,493–16,510 (1989).
27. McCormick, M. P. & Trepte, C. R. *J. geophys. Res.* **92,** 4297–4306 (1987).
28. Hamill, P., Turco, R. P. & Toon, O. B. *J. atmos. Chem.* **7,** 287–315 (1988).
29. McCormick, M. P., Trepte, C. R. & Pitts, M. C. *J. geophys. Res.* **94,** 11,241–11,251 (1989).
30. Poole, L. R., Solomon, S., McCormick, M. P. & Pitts, M. C. *Geophys. Res. Lett.* **16,** 1157–1160 (1989).

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Geometry of subduction and depth of the seismogenic zone in the Guerrero gap, Mexico

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THE western coast of Guerrero in southern Mexico has been identified as a seismic gap on the Middle American Trench in which no large earthquakes have occurred at least since 1908. It has been suggested that the seismic energy accumulated since 1908 will eventually be released by a large earthquake. A permanent seismic network was installed to monitor the seismicity of this mature seismic gap and to understand the geometry of the subducted slab beneath this region. The seismicity defines an unusual distribution along two bands of seismic activity parallel to the coast. The resulting geometry of the subduction zone shows that the Cocos plate dips at a shallow angle beneath the North American plate to a depth of ~40 km; from there the subducted slab is bent upward, following a subhorizontal trajectory extending inland at a depth of ~50 km. This plate geometry is reminiscent of that found in Peru and central Argentina, two other regions where a young oceanic plate is being subducted. In Mexico, however, the slab underplates an overriding plate which is only half as thick as that observed in South America. A possible oceanic origin of the allochtonous terranes comprising southern Mexico may explain the presence of the anomalously thin lithosphere in this region.

The Guerrero gap in southern Mexico is perhaps one of the more clearly identified seismic gaps in the circum-Pacific belt[1-3]. It lies immediately south of the rupture area of the 1985 Michoacan earthquake[4,5] (Fig. 1). A telemetered, nine-station seismic network northwest of Acapulco (Fig. 1) records daily an average of four to five earthquakes, with coda-wave magnitude (M_c) in the range 1-4, which are located using the program HYPO71 (ref. 6) and a velocity model reported for this area[7]. The results presented here include events recorded during a three-month temporary experiment performed in 1986 and earthquakes located from August 1987 to December 1988 by the permanent network.

The distribution of seismicity shows an unusual disposition along two bands of activity (Fig. 1). The coastal band of seismicity is ~35 km wide and shows hypocentres with focal depths of between 10 and 25 km. The second zone of seismicity lies farther inland and is clearly separated from the coastal activity, showing focal depths of between 32 and 42 km. The absence of earthquakes between these two seismic bands is more evident where the station coverage is best. Also, practically no seismic activity is located between the coastal seismic zone and the trench (Fig. 1). Although this area is outside the network, the absence of seismic activity here is not due to its distance to the seismic instruments on land; small earthquakes ($0 < M_c < 1$) are routinely located along the coast at equivalent distances from the centre of the network. This low level of near-trench seismicity appears where saturated sediments may have a dominant role in the mechanical behaviour of the plate interface.

In cross-section, the hypocentral distribution reflects the subduction of the Cocos plate beneath Mexico (Fig. 2). The void of seismicity between depths of 25 and 32 km, separating the two parallel seismic zones, is clearly visible on cross-sections where the accuracy of the locations is better (BB′, CC′ and DD′). The apparent absence of this void in section AA′ probably reflects poor hypocentral control of earthquakes located outside

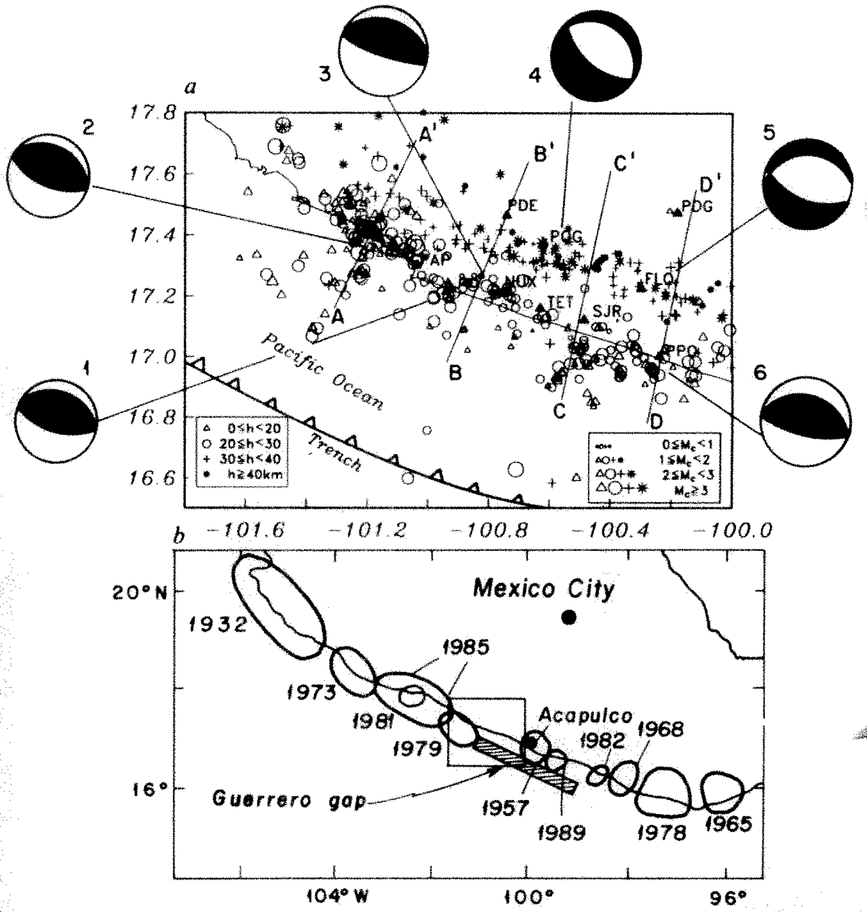

FIG. 1 *a*, Map showing the location of stations of the Guerrero seismic network (solid triangles) and of the selected epicentres. Cross-sections are shown in Fig. 2. Composite fault-plane solutions are in lower-hemispheric projection; solid quadrants indicate compressional first motions. *b*, Location of the Guerrero gap relative to the rupture areas (ovals) of previous large earthquakes in the region. Station coordinates: PDE, 17.46° N, 100.74° W; TET, 17.16° N, 100.63° W; NUX, 17.21° N, 100.75° W; PAP, 17.30° N, 101.04° W; POP, 17.02° N, 100.24° W; FLO, 17.22° N, 100.39° W; POG, 17.37° N, 100.62° W; PDG, 17.47° N, 100.18° W; SJR, 17.14° N, 100.47° W.

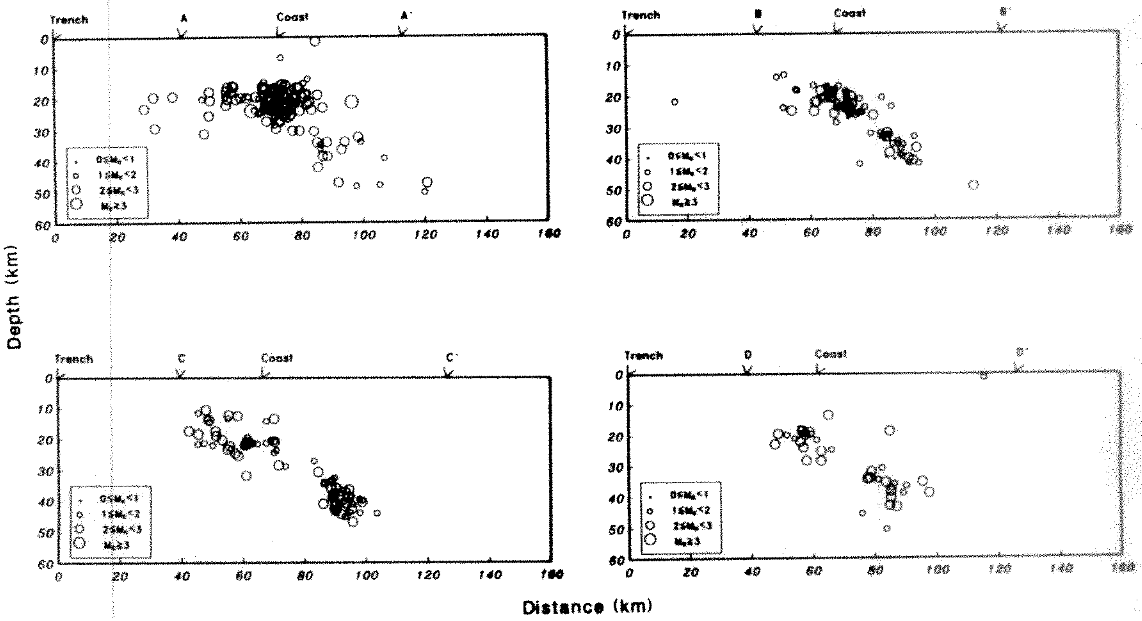

FIG. 2 Cross-sections of the seismicity (open circles) shown on Fig. 1. Size of symbols indicate magnitude of hypocentres. Notice the two distinct seismic clusters in the three cross-sections made within the seismic network (BB', CC' and DD').

of the network. Notice also that the overriding plate is virtually aseismic (Fig. 2). Composite fault-plane solutions of the coastal earthquakes show shallow-thrust faulting dipping to the northeast, reflecting the relative motion of the Cocos and North American plates (Figs 1 and 3). On the other hand, the deeper seismic zone shows predominantly normal faulting with tensional axes almost horizontal and oriented in the direction of relative plate motion (Figs 1 and 3).

No microseismicity was located inland of the second seismic band. Because of this, the trajectory of the subducted slab farther inland can not be determined only from the microearthquakes located by the network. Nevertheless, some large events occurring inland have been studied using teleseismic data, such as the Tlapehuala event of 6 June 1964 ($M_s = 6.7$). Its focal mechanism and hypocentral depth are well constrained using body-wave modelling[8], indicating normal faulting with almost horizontal T-axis oriented NNE–SSW (Fig. 4) at a depth of 55 km, similar to that of other tensional earthquakes within the subducted slab in central Mexico[9–13]. Another earthquake on 2 July 1968 also shows (ref. 14) normal faulting at a depth of 45 km[14] in the region where no seismicity has been recorded by the network. The geometry of the slab is inferred by projecting the hypocentre of these two earthquakes onto a cross-section together with the microseismic data (Fig. 4).

The data show that the Cocos plate subducts beneath the North American plate at a shallow angle which steepens progressively to ~12°. The maximum depth of strong seismogenic plate contact is 25 km. Beneath this depth the subducted slab bends sharply, following a quasi-horizontal trajectory underplating southern Mexico for at least 150 km (Fig. 4). The narrow coastal seismicity reflects the relative motion of the Cocos and North American plates, whereas the tensional earthquakes located deeper and farther inland seem to reflect flexural stresses induced by the sharp bend in the slab. The morphology of the slab to the north of the flat portion is still not known (Fig. 4). Probably the slab steepens north of this region, reaching a depth of ~100 km beneath the volcanic belt, as observed in most subduction zones[15]. The apparent buoyancy of the slab in central Mexico may be due to the young age of the Cocos plate. In southeastern Mexico and Central America, where the Cocos plate is older[16], the slab dip becomes steeper (30°) with no evidence of a horizontal segment[10–12].

The flexure of the subducted slab near the coast may explain also the presence of great, lithospheric tensional earthquakes occurring in Mexico immediately downdip of the megathrust plate contact. The 15 January 1931 event in Oaxaca ($M_w = 8.0$)[13], and probably the great earthquake of 19 June 1858 in northern Michoacan ($M_w \approx 8.0$), are difficult to explain by the purported gravitational pull of a slab that is subhorizontal and shallower than ~100 km in central Mexico[11,12]. Their origin is perhaps better explained by flexural stresses present in the contorted slab.

This bend in the slab at a depth of ~30 km apparently limits

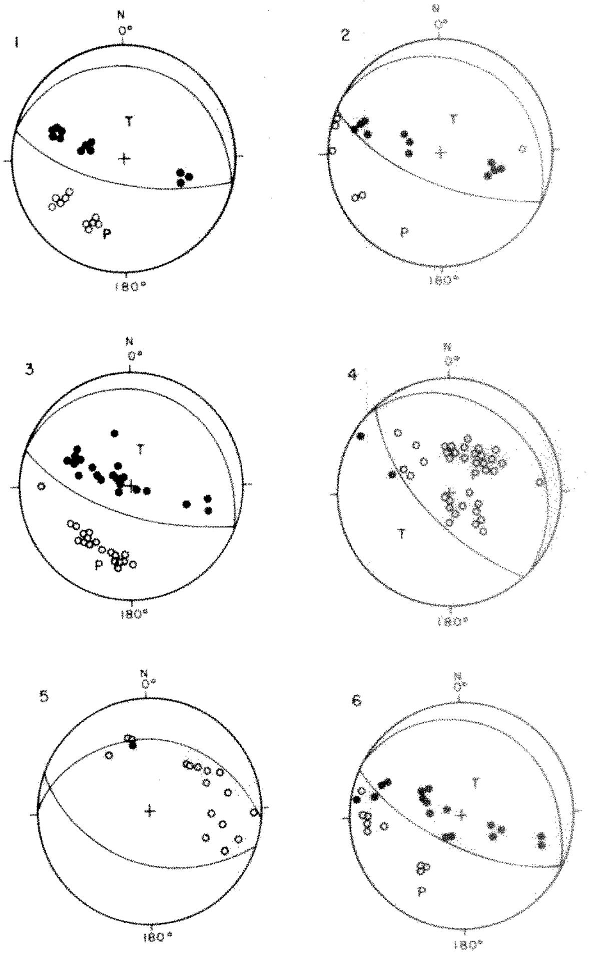

FIG. 3 Composite focal mechanisms of the earthquakes shown on Fig. 1. Solid circles indicate compressional first motions; smaller symbols are indicative of nodal arrivals. The letters P and T indicate axes of maximum and minimum compression respectively. Projection is on a lower-hemisphere stereonet.

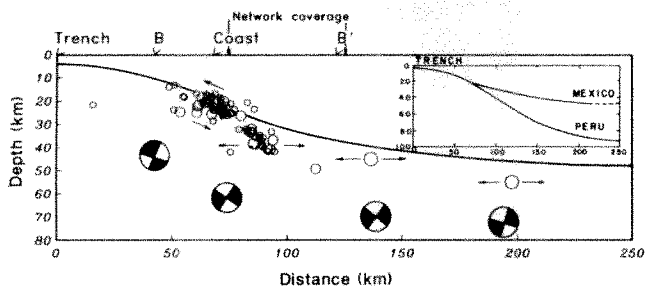

FIG. 4 Geometry of the subducted slab beneath southern Mexico. Seismicity corresponds to that of cross-section CC′ on Figs 1 and 2. Focal mechanisms of microearthquakes and of teleseismic events[8,14] are shown on side-looking, lower-hemisphere projection. Dark quadrants indicate compressional first motions. Arrows indicate approximate projected orientation of the T-axes. Insert shows comparison between subduction zones in central Peru[22,23] and southern Mexico. Notice the continental lithosphere in Peru is twice as thick as that of Mexico.

the downdip extent of the seismogenic zone, explaining the unusually narrow megathrust plate contact found in the Mexican subduction zone; a fact corroborated by aftershock studies performed after large subduction earthquakes in Mexico[4,5,7,17-19], which show that the rupture areas of interplate events in this region are consistently shallower than 20 km. This relatively narrow seismogenic interface in Mexico may explain why the Middle American arc, where young oceanic lithosphere is being subducted at a fast rate ($\sim$6.5 cm yr^{-1}), does not have the large contact zone that results in major earthquakes ($M_w > 9.0$) like those of 1960 in Chile or 1964 in Alaska. The seismogenic zone in Guerrero is as narrow as the 1985 Michoacan rupture. Thus, the length of the northwestern Guerrero gap would result in an earthquake of the same magnitude as the 1985 event ($M_w = 8.1$). If the rupture propagates south of Acapulco, the magnitude could be as high as 8.4.

The plate geometry in Guerrero is reminiscent of that observed in central Peru[20-23] and Argentina[12,20,24], where the Nazca plate lies subhorizontally, underplating the South American plate. central Peru, Argentina, and central Mexico are apparently the only three subduction zones that exhibit a horizontal slab beneath the upper continental plate. However, the main difference between the subduction in Mexico and those of South America is the lithospheric thickness of the overriding plate. Locally recorded microearthquakes[21-23,25] and accurate depth determinations of teleseismic events[22,24], show the horizontal seismic zone beneath central Peru and Argentina lies at an average depth of 100 km, which corresponds to a lithospheric thickness of ~90 km. In contrast, the average lithospheric thickness in southern Mexico is only ~45 km (Fig. 4).

This thin lithosphere in southern Mexico is more similar to that of an oceanic plate[26] than to the thicker continental lithosphere[27] expected in this region. This is probably explained by the fact that southern Mexico was formed by the accretion of allochtonous terranes onto the continent since the Palaeozoic[28-31]. The thin lithosphere underlying these allochtonous terrances suggests they may be oceanic in nature. Otherwise, the lithosphere in southern Mexico could be originally continental and later thinned by an extensional phase of deformation when swarms of mafic dykes were intruded[29,32]. In any case, the young and presumably buoyant subducted slab seems simply to follow the lower topography of this thin overriding plate. ☐

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1. Kelleher, J., Sykes, L. & Oliver, J. *J. geophys. Res.* **78,** 2547–2585 (1973).
2. McNally, K. & Minster, J. B. *J. geophys. Res.* **86,** 4949–4959 (1981).
3. Nishenko, S. & Singh, S. K. *Bull. seism. Soc. Am.* **77,** 2095–2114 (1987).
4. UNAM Seismology Group *Geophys. Res. Lett.* **13,** 573–576 (1986).
5. Stolte, C. *et al. Geophys. Res. Lett.* **13,** 577–580 (1986).
6. Lee, W. H. K. & Lahr, J. C. *U.S. Geol. Surv., Open File Report* 75–311 (1975).
7. Valdes, C., Meyer, R. P., Zuñiga, R., Havskov, J. & Singh, S. K. *J. geophys. Res.* **87,** 8519–8527 (1982).
8. González-Ruíz, J. thesis, Univ. of California, Santa Cruz (1986).
9. Jimenez, Z. & Ponce, L. *Geofis. Int.* **17,** 379–386 (1979).
10. Nixon, G. T. *Bull. geol. Soc. Am.* **93,** 514–523 (1982).
11. Burbach, G. V., Frohlich, C., Pennington, W. D. & Matsumoto, T. *J. geophys. Res.* **89,** 7719–7735 (1984).
12. Bevis, M. & Isacks, B. *J. geophys. Res.* **89,** 6153–6170 (1984).
13. Singh, S. K., Suárez, G. & Dominguez, T. *Nature* **317,** 56–58 (1985).
14. Molnar, P. & Sykes, L. R. *Bull. geol. Soc. Am.* **80,** 1639–1684 (1969).
15. Isacks, B. & Barazangi, M. *Maurice Ewing Series* **1,** *Am. Geophys. Union,* 99–114 (1977).
16. Couch, R. & Woodcock, S. *J. geophys. Res.* **86,** 1829–1840 (1981).
17. Ponce, L., McNally, K. C., González, J., Del Castillo, L. & Chael, E. *Geofis. Int.* **17,** 267–280 (1979).
18. Singh, S. K. *et al. Science* **207,** 1211–1213 (1980).
19. Havskov, J., Singh, S. K., Nava, E., Dominguez, T. & Rodriguez, M. *Bull. seism. Soc. Am.* **73,** 449–458 (1983).
20. Barazangi, M. & Isacks, B. *Geophys. J. R. astr. Soc.* **57,** 537–555 (1979).
21. Grange, F. *et al. J. geophys. Res.* **89,** 6139–6152 (1984).
22. Hasegawa, A. & Sacks, I. S. *J. geophys. Res.* **86,** 4971–4980 (1981).
23. Suárez, G. *et al. Geophys. J.* (in the press).
24. Araujo, M. & Suárez, G. *J. geophys. Res.* (submitted).
25. Grange, F. *et al. Geophys. Res. Lett.* **11,** 38–41 (1982).
26. Forsyth, D. *Geophys. J. R. astr. Soc.* **43,** 103–162 (1975).
27. Press, F. *J. geophys. Res.* **75,** 6575–6581 (1970).
28. Campa, M. F. & Coney, P. *Can. J. Earth Sci.* **26,** 1040–1051 (1983).
29. Ortega-Gutiérrez, F. *Geofis. Int.* **20,** 177–202 (1981).
30. Pantoja-Alor, J. & Estrada-Barraza, M. *Bol. Soc. geol. Mex.* **48,** 1–15 (1986).
31. Gastil, G., Krumenacher, D. & Jensky, W. A. *Geol. Soc. Am. Map Chart Ser.,* **M24,** (1978).
32. Cserna, Z. *Bol.* **62,** Inst. Geol., Univ. Nac. Auton. Mex. (1965).

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Implications of a new acoustic target strength for abundance estimates of Antarctic krill

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ANTARCTIC krill (*Euphausia superba*) is the dominant component of the diet of many whales, seals, birds, fish and squid, and their survival could be affected by a reduction in krill abundance due to fishing[1]. Commercial fishing takes nearly half a million tonnes of krill annually[2] and accurate estimates of abundance are needed for rational management of this resource. Indirect estimates of abundance based on predator consumption rates give a roughly estimated total annual production of several hundred million tonnes[3]. The life-span of krill is at least two and maybe five years, so the standing stock would need to be of at least the same magnitude as the annual production. But direct estimates of abundance using nets and acoustics have indicated biomass figures lower by an order of magnitude[3]. Acoustic estimates are sensitive to the scaling factor or target strength (TS) used to convert echo energy to absolute abundance. Previous published values for TS (ref. 4), when applied to survey data, gave estimates of krill abundance that were much too low to account for local bird and seal predation rates near South Georgia[5], and were also lower than expected when compared with density estimates from net hauls[6]. We therefore sought to determine TS using direct measurements developed for fish studies[7], and also by applying models developed for other crustacean zooplankton[8]. Our results show that krill TS is much lower than previously thought, and consequently that acoustic estimates of krill abundance are likely to have been gross underestimates.

The field study was undertaken in Stromness Harbour, South Georgia; a comprehensive description of the methodology is published elsewhere[9]. Mean TS values for groups of krill were

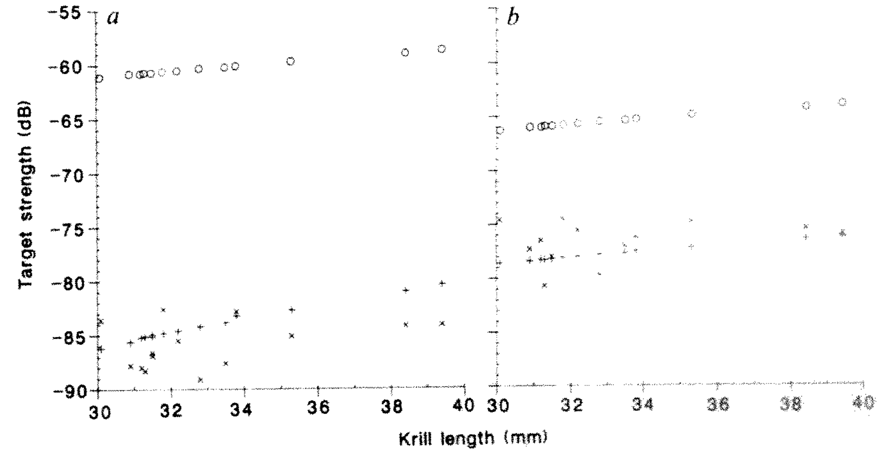

FIG. 1 A comparison of TS values at 38 kHz (*a*) and 120 kHz (*b*) derived from cage experiments (×), theoretical modelling (+) and from the BIOMASS equation (○) (ref. 4). TS in cage experiments was calculated by the subtraction method (see also Table 1). For the sound-scattering model[8] a mean density of 1.0647 ± 0.0069 g cm^{-3} for krill of mean length 31 mm was used, and a mean sound speed contrast of 1.0279 ± 0.0024 was obtained by measuring the time delay for a sound pulse to travel through the tranverse piece of an inverted T-shaped tube with ceramic transducers, resonant at 500 kHz, mounted at each end[13]. Measurements were made using clean sea water and then with increasing numbers of krill until the T-tube was more or less full. Previously used values of TS are derived from BIOMASS[4]. At 38 kHz the mean value for the model is 2.5 dB higher than the mean value of the cage experiment (s.e. 0.35, t 7.0, $P < 0.001$). At 120 kHz the mean value for the model is 0.7 dB lower than the experiment (s.e. 0.52, t 1.4, P 0.2).

obtained by confining known numbers of krill in a nylon mesh cage suspended from a moored raft. Downward-directed echo sounder transducers operating at 38 and 120 kHz were aimed at the cage (0.5 m high and 0.5 m diameter). Up to 1,600 krill were placed in the cage producing densities as high as 16,000 m^{-3}, well within the range reported for naturally occurring swarms[3]. Additional experiments were carried out with no krill in the cage. The transducers were connected to a Simrad EK400 echo sounder, Simrad QD digital integrator and data logger. The echo sounder transceivers were periodically calibrated and transducer beam patterns measured by the moving sphere method[10]. A tungsten carbide sphere, positioned close to the main acoustic axes of both transducers and above the cage, provided a continuous check on system stability.

Experiments were run for 15–64 h and the mean echo level recorded every 6 min. The TS that was determined, first by subtraction of echo energy of cage and krill (Table 1), and second by regression of echo energy against krill number (Table 1), is very much lower than calculated values using equations produced by the Biological Investigation of Marine Antarctic Systems and Stocks (BIOMASS) programme (Table 1). At 120 kHz the values are at least 10 dB higher than our experimental ones. Although results at 38 and 50 kHz are not directly comparable, a difference of 25 to 30 dB indicates a considerable inconsistency between the two.

TABLE 1 Summary results of TS of *Euphausia superba* from cage experiments calculated by the methods of subtraction and regression and compared with TS calculated from BIOMASS[4]

Frequency (kHz)	Method	cv (%)	$\overline{TS}$	$\overline{TS} - s.d.$	$\overline{TS} + s.d.$
120	Subtraction	31	−76.1	−77.7	−74.9
	Regression	33	−78.6	−80.3	−77.4
	BIOMASS		−65.9		
38	Subtraction	47	−85.1	−87.9	−83.4
	Regression	46	−89.4	−92.1	−87.7
50	BIOMASS		−60.4		

In the subtraction method $TS = 10 \log ((I_k - I_c)/N)$ where I_k is the relative echo energy due to the cage and krill, I_c the echo energy due to the cage alone, and N is the number of krill in the cage. This approach assumes that the echo energy due to a group of scatterers is equal to the sum of the individual scatterer contributions to the echo energy. In the regression method the single krill target strength was estimated from the least squares regression of I_k on N for all experiments. Mean length of krill in the experiments ranges from 29.6 mm to 38.9 mm, overall mean is 32.6 mm. cv(%), Coefficient of variation.

The difference between the TS values calculated by subtraction and regression is small relative to the difference between these and the BIOMASS values[4]. It is still nonetheless disconcertingly large. This may be due in part to the relatively large and variable contribution of the empty cage. Subsidiary causes of variability may be due to the degree of aggregation, orientation and size of krill. Video recordings of the krill revealed frequent concentration of the animals into a small fraction of the cage, even at the highest densities used. While some of this behaviour was probably induced by water flow through the cage, the general trend of daytime concentration and dusk dispersal was similar to behaviour observed in the open ocean[11]. The dominant characteristics affecting TS of marine organisms are gas inclusions, which krill do not possess, and contrasts in density, longitudinal-wave sound speed, and compressibility with respect to sea water. Using the scattering model of Greenlaw[8] we calculated a theoretical TS and compared it with the subtraction values from the cage experiments (Fig. 1). Although agreement between model and experimental values at 120 kHz is greater than at 38 kHz, the model values are very much closer to our experimental values than to the BIOMASS values (Fig. 1). The model is by necessity oversimplified with respect to recent models[12] in which orientation has a key role, and further study on orientation, degree of aggregation, and biological condition is likely to be most fruitful in connecting measurement and theory.

The BIOMASS equations[4] show a marked change in TS with krill size. Unfortunately the size spectrum of krill used in our experiments was very small (23–45 mm). Krill may grow to more than 60 mm long[3] so our results represent a small part of the overall size spectrum of krill present in the wild. We therefore consider it inadvisable to extrapolate our results to provide TS-to-size relationship over the full size range of krill. Both the implications of these results are clear: values of TS used previously are far too high and standing stock estimates which have relied on them underestimate krill biomass by at least an order of magnitude. We believe that the results reported here, although having some limitations due to the small size range of krill available for the study, provide a far more reliable basis for future estimates of biomass. □

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1. Laws, R. M. *Am. Sci.* **73,** 26–40 (1985).
2. Report of the Seventh Meeting of the Scientific Committee of the Commission for the Conservation of Antarctic Marine Living Resources, SC-CAMLR-VII, XXXX (CCAMLR, Hobart, 1988).
3. Miller, D. G. M. & Hampton, I. *BIOMASS Sci. Ser.* **9,** (1989).
4. Report on Post-FIBEX Acoustic Workshop, Frankfurt, Federal Republic of Germany, *BIOMASS Report Series* No. 40 (1986).
5. Croxall, J. P., Prince, P. A. & Ricketts, C. in *Antarctic Nutrient Cycles and Food Webs* (ed. Siegfried, W. R., Condy, P. R. & Laws, R. M.) 516–533 (Springer, Berlin, 1985).

6. Everson, I. & Bone, D. G. *Polar Biol.* **6,** 83–90 (1986).
7. Foote, K. G. *J. Acoust. Soc. Am.* **73,** 1932–1940 (1983).
8. Greenlaw, C. F. *Limnol. Oceanogr.* **24,** 226–242 (1979).
9. Foote, K. G., Everson, I., Watkins, J. L. & Bone, D. G. *J. Acoust. Soc. Am.* **87,** 16–24 (1990).
10. Foote, K. G., Knudsen, H. P., Vestnes, G., MacLennan, D. N. & Simmonds, E. J. *Calibration of Acoustic Instruments for Fish Density Estimation: A Practical Guide* (ICES, Copenhagen, 1987).
11. Everson, I. *J. Plankton. Res.* **4**(1), 155–162 (1982).
12. Stanton, T. K. *J. Acoust. Soc. Am.* **83,** 55–63 (1988).
13. Kogeler, J. W., Falk-Petersen, S., Kristensen, A., Pettersen, F. & Dalen, J. *Polar Biol.* **7,** 231–235 (1987).

Gliding behaviour and palaeoecology of the alleged primate family Paromomyidae (Mammalia, Dermoptera)

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THE extinct Paromomyidae, known from early Tertiary strata of North America[1–6] and Europe[7–8], have traditionally been viewed as an archaic radiation of the order Primates[1–11]. Hypotheses about the phylogenetic position and palaeobiology of these animals have, however, been highly constrained because postcranial fossils of paromomyids have not been positively identified until now. Newly discovered fossils representing the paromomyid genera *Phenacolemur* and *Ignacius* show that these animals share functionally important postcranial synapomorphies with extant *Cynocephalus* (the flying lemur), the only living member of the mammalian order Dermoptera. These traits strongly suggest that paromomyids possessed a patagium, or gliding membrane, which was homologous to that of *Cynocephalus*. Paromomyids therefore provide both the earliest evidence for the evolution of gliding behaviour in mammals and the only fossil record currently recognized for the eutherian order Dermoptera[12].

Two dentally associated partial skeletons of the paromomyid genus *Phenacolemur* have recently been recovered from early Eocene (Wasatchian) rocks in the Bighorn and Clark's Fork Basins of northwestern Wyoming. These specimens, UM 66440 and UM 86352 (which represent a single individual of *P. praecox*) and USGS 17847, have allowed the identification of additional, dentally unassociated postcranial fossils representing both *Phenacolemur* and the closely related paromomyid *Ignacius* from an early Wasatchian faunule in the Clark's Fork Basin[13,14].

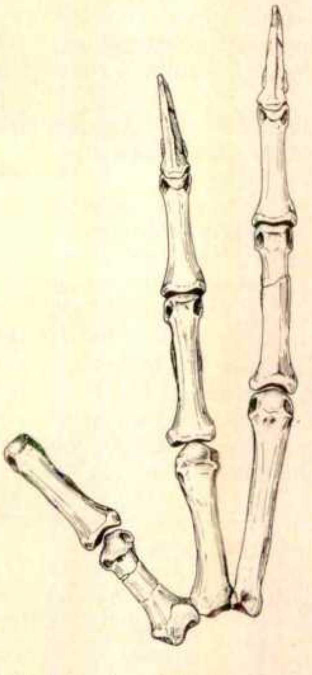

FIG. 1 Right partial hand skeleton of the plesiadapid *Nannodectes intermedius* (USNM 442229). Note relative proportions of phalangeal elements.

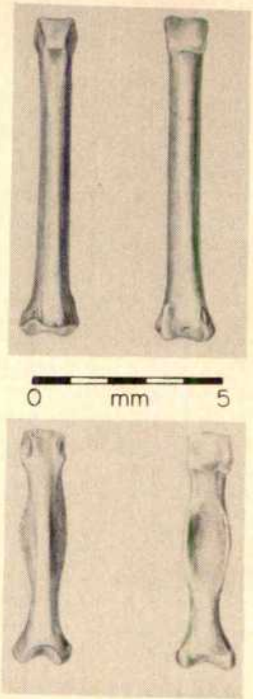

FIG. 2 Proximal (bottom; USNM 442248) and intermediate (top; USNM 442254) manual phalanges of the paromomyid *Phenacolemur simonsi*.

Perhaps the most unusual aspect of the postcranial anatomy of paromomyids revealed by the new specimens is the osteology of the manus and pes. One way in which paromomyids differ from plesiadapids (believed to be closely related to paromomyids on the basis of craniodental morphology[4,9–10]) is in possessing intermediate phalanges that are much longer than their corresponding proximal phalanges (Figs 1–3). This very uncommon condition is found among living mammals only in the dermopteran *Cynocephalus* and in edentates (sloths, armadillos and anteaters) (Fig. 3). The overall anatomy of the phalanges of edentates is, however, very different from that of *Cynocephalus* and paromomyids, and their unusual phalangeal proportions seem to result from their peculiarly stub-like proximal phalanges, rather than from elongation of the intermediate phalanges *per se* (Fig. 3). By contrast, phalangeal morphology is extremely similar in paromomyids and extant *Cynocephalus*.

Given this close anatomical similarity, the functional significance of the greatly elongated intermediate phalanges of paromomyids can be assessed in light of the anatomy of extant *Cynocephalus*. In *Cynocephalus* the patagium extends between the individual digits of both the manus and the pes, so that pronounced interdigital webbing of the patagium occurs from the level of the metacarpo- or metatarsophalangeal joints (proximally) to the level of the distal interphalangeal joints (distally)[15]. Although the capacity for gliding has evolved independently at least six times in mammals, only in *Cynocephalus* does the patagium form this interdigital webbing[16]; for this reason *Cynocephalus* has been described as a 'mitten glider'[16].

Because of the interdigital webbing of the patagium in *Cynocephalus*, the degree of flexion-extension of the proximal interphalangeal joints, especially of the manus, should have a direct effect on the amount of billowing to which the patagium is subjected. The great elongation of the intermediate phalanges in *Cynocephalus* amplifies this relationship, and therefore seems to be functionally related to enhancing voluntary control over the degree of patagial billowing. The latter factor greatly influences the aerodynamics of gliding in mammals[17].

The new evidence presented here strongly indicates that paromomyids also possessed a patagium with significant interdigital webbing, as occurs only in *Cynocephalus* among extant mammalian gliders. As noted previously, plesiadapids lack the specializations in phalangeal anatomy shared by paromomyids and *Cynocephalus*. Therefore, there is no evidence that plesiadapids possessed a patagium. In a phylogenetic context, the evolution of a patagium for gliding locomotion and the osteological features that are functionally associated with this development imply that paromomyids comprise the sister group of *Cynocephalus*, thus forming a clade that excludes the family Plesiadapidae (Fig. 4). Further postcranial synapomorphies also link paromomyids with extant *Cynocephalus*[13]. These traits include a major reorganization of the proximal carpus, in which the lunate is positioned distal to the scaphoid rather than ulnar to it (thus allowing the triquetrum to articulate with both lunate and scaphoid on its radial aspect), and strong development of a secondary synovial articulation between the posterior side of the sustentaculum tali and the plantar aspect of the medial astragalar pillar. Although these and other postcranial characters[13] provide substantial additional support for the phylogenetic relationships depicted in Fig. 4, they are not discussed further here because they have no direct functional relationship with gliding behaviour.

In addition to forming the basis for this novel phylogenetic hypothesis, the new postcranial fossils of paromomyids provide fresh insight into their palaeobiology. For example, it has been known for some time that the dentition of paromomyids is strongly convergent on that of the extant sugar-glider *Petaurus*[18-20], a phalangeroid marsupial that feeds extensively on tree exudates[21,22]. The fossil evidence reported here suggests that paromomyids were a much closer ecological analogue to extant *Petaurus* than had previously been imagined, as they apparently evolved a patagium convergent on that of extant sugar-gliders in addition to the dental features noted previously.

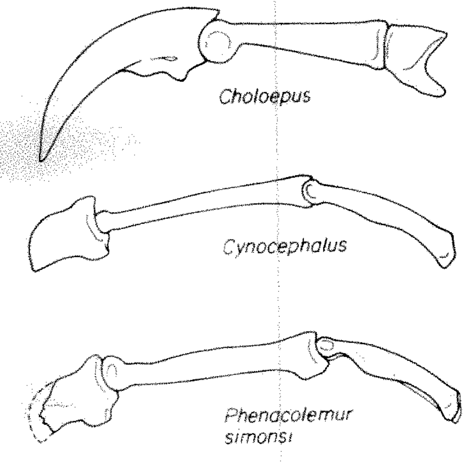

FIG. 3 Manual phalanges of the edentate *Choloepus* (two-toed sloth), the dermopteran *Cynocephalus* (flying lemur), and the paromomyid *Phenacolemur* in lateral view (not to same scale). Compare with Fig. 1.

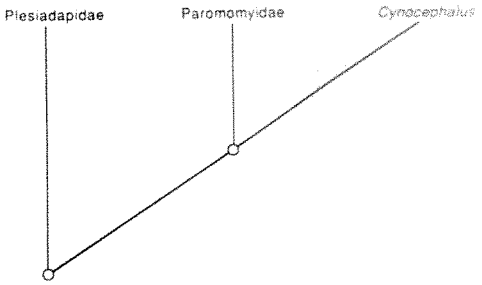

FIG. 4 Cladogram illustrating phylogenetic position of Paromomyidae, based on characters discussed in the text. Note that the possible sister-group relationship between Plesiadapidae and a clade comprising Paromomyidae and *Cynocephalus* has not been established here (but see ref. 13); rather, plesiadapids are simply used as an outgroup for the latter clade here.

The preceding hypothesis is corroborated by the pattern of individual abundance and stratigraphic longevity shown by paromomyids. Unlike plesiadapids, which often formed a dominant component of Palaeocene mammalian assemblages in both Europe[23] and North America[24,25], paromomyids were never very abundant, as might be expected of animals with a highly specialized ecological niche. Likewise, while plesiadapids became extinct near the Palaeocene-Eocene boundary in North America[6,26], paromomyids persisted on that continent into the Duchesnean Land-Mammal Age (late Eocene)[5,27]. It has been suggested[26] that the extinction of plesiadapids in North America may have resulted from competitive exclusion by early rodents, which immigrated into North America from Asia at the beginning of Clarkforkian time (late Palaeocene)[28]. The ecological specializations of paromomyids discussed here evidently resulted in minimal competition between paromomyids and early rodents, allowing the two taxa to coexist in North America for some 18 million years[29]. The eventual extinction of paromyids in North America occurred near the Eocene-Oligocene boundary, at which time rapid climatic and floral changes[30] probably resulted in the deterioration of habitats required by such obligatory forest-dwelling mammals. ☐

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1. Matthew, W. D. *Bull. Am. Mus. nat. Hist.* **34,** 429–483 (1915).
2. Gidley, J. W. *Proc. US natn. Mus.* **63**, 1–38 (1923).
3. Simpson, G. G. *Bull. Am. Mus. nat. Hist.* **105,** 411–442 (1955).
4. Bown, T. M. & Rose, K. D. *Folia primatol.* **26,** 109–138 (1976).
5. Krishtalka, L. *Ann. Carnegie Mus.* **47,** 335–360 (1978).
6. Rose, K. D. & Bown, T. M. *J. vert. Paleont.* **2,** 63–69 (1982).
7. Russell, D. E., Louis, P. & Savage, D. E. *Univ. Calif. Publs. geol. Sci.* **73,** 1–46 (1967).
8. Godinot, M. *Folia primatol.* **43,** 84–96 (1984).
9. Szalay, F. S. & Delson, E. *Evolutionary History of the Primates* (Academic, New York, 1979).
10. Szalay, F. S., Rosenberger, A. L. & Dagosto, M. *Yb. phys. Anthrop.* **30,** 75–105 (1987).
11. Szalay, F. S. *Am. J. phys. Anthrop.* **36,** 59–76 (1972).
12. MacPhee, R. D. E., Cartmill, M. & Rose, K. D. *J. vert. Paleont.* **9,** 329–349 (1989)
13. Beard, K. C. thesis, The Johns Hopkins University, Baltimore, Maryland (1989)
14. Beard, K. C. & Houde, P. *J. vert. Paleont.* **9**, 388–399 (1989).
15. Pocock, R. I. *Proc. zool. Soc. Lond.* 429–444 (1926).
16. Thorington, R. W., Jr *Science* **225,** 1048–1050 (1984).
17. Thorington, R. W., Jr & Heaney, L. R. *J. Mammal.* **62,** 101–114 (1981).
18. Gingerich, P. D. *J. dent. Res.* **53**, 497 (1974).
19. Cartmill, M. *Science* **184**, 436–443 (1974).
20. Kay, R. F. & Cartmill, M. *J. hum. Evol.* **6,** 19–53 (1977).
21. Wakefield, N. A. *Vict. Nat.* **87,** 221–236 (1970).
22. Smith, A. P. *J. Anim. Ecol.* **51,** 149–166 (1982).
23. Russell, D. E. *Mém. Mus. natn. Hist. nat.* (*Sér. C*) **13,** 1–324 (1964).
24. Gingerich, P. D. *Univ. Mich. Pap. Paleont.* **15,** 1–141 (1976).
25. Rose, K. D. *Univ. Mich. Pap. Paleont.*, **26,** 1–197 (1981).
26. Maas, M. C., Krause, D. W. & Strait, S. G. *Paleobiology* **14,** 410–431 (1988).
27. Storer, J. E. *Dakoterra* **3,** 108–117 (1987).
28. Krause, D. W. *Univ. Wyoming Contr. Geol. spec. Pap.* **3,** 95–117 (1986).
29. Berggren, W. A., Kent, D. V., Flynn, J. J. & Van Couvering, J. A. *Geol. Soc. Am. Bull.* **96,** 1407–1418 (1985).
30. Wing, S. L. *Ann. Missouri Bot. Gard.* **74,** 748–784 (1987).

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Eocene plesiadapiform shows affinities with flying lemurs not primates

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PLESIADAPIFORMES, of the North American and European Paleogene, is often identified as a sister group of primates. This hypothesis is based on several proposed anatomical synapomorphies linking the best-known plesiadapiform families, Plesiadapidae, and Paromomyidae with Eocene primates[1-5]. The first well-preserved skull of *Ignacius graybullianus*, an early Eocene paromomyid plesiadapiform, clarifies and corrects previous cranial reconstruction based on more fragmentary material[3,6,7]. The new material indicates Plesiadapiformes are not Primates. Rather, several synapomorphies argue for a closer phylogenetic relationship between Plesiadapiformes and *Cynocephalus*, the extant flying lemur (order Dermoptera). In view of the finding that "archaic" primates are not cladistic Primates, the recently coined taxon "Euprimates" should be discarded. No support is lent by cranial anatomy to the hypothesis that Primates, tree shrews, bats and dermopterans form a clade Archonta.

The new specimen of *Ignacius graybullianus*, USNM 421608, is a nearly complete skull collected by P. Houde from early Wasatchian (Wa_1, earliest Eocene[8]) rocks of the Willwood Formation, Clark's Fork Basin, Park County, Wyoming. The USNM 421608 skull (Figs 1 and 2) is slightly crushed interorbitally and the face is slightly displaced dorsally relative to the braincase. The premaxillae were lost during deposition, but the rest of the skull, including the basicranium, is complete. Here we focus on the basicranial anatomy considered crucial for phylogenetic inference. Several plesiadapiform basicrania have been reported previously. These include poorly preserved material of the paromomyids *Ignacius*[6] and *Phenacolemur*[3], and several better preserved specimens of the plesiadapid *Plesiadapis*[1,2].

The large auditory bulla is completely ossified (Figs 1-3). It is greatly expanded medially and balloons caudally over the basioccipital. The bulla is composed of the entotympanic, not of the basisphenoid, basioccipital, ectotympanic, or, as in primates, the petrosal[5,9,10]. This is evidenced by the following observations: (1) sutures separate the medial, rostral and caudal bullar walls from the basisphenoid and basioccipital; (2) laterally, sutures are visible between the collar-shaped ectotympanic and the bulla; (3) inside the right bulla on the roof of the tympanic cavity, the promontorial part of the petrosal is separated from the bulla medially by a suture. That this is a suture and not a fracture or other artefact is demonstrated by structure and symmetry. This suture is formed by an overlapping of the petrosal element onto the bone of the bulla. The bones thin out at their junction rather than butting bluntly against one another as might appear in postmortem breakage. Furthermore, the position of the suture is identical on both sides of the skull as viewed directly ventrally on the right side (Fig. 3) and through a rostromedial break in the left bulla. The bulla of *Plesiadapis* may have formed in a similar manner followed by full co-ossification of the entotympanic-petrosal suture[11].

Having an auditory bulla formed wholly or in part by an entotympanic is a key distinction between Plesiadapiformes and Primates, which have a petrosal bulla[5,9]. If the bulla was membranous in primitive eutherians[12,13], the presence of an entotympanic bulla in *Ignacius* would be a derived character that rules *Ignacius* and other Plesiadapiformes out of the ancestry of Primates. Bullar entotympanics have evolved in many mammalian groups[10]. However, an unusual similarity in the entotympanics of *Ignacius* and the living dermopteran *Cynocephalus* is that the entotympanic contacts the basioccipital ventromedially[14]. This is a distinction from the entotympanics of tree shrews, for example, in which the only bony contact medially is with the petrosal.

Previous proposals of a significant intracranial blood supply for paromomyids by way of either the internal carotid artery[3,9] or the ascending pharyngeal artery[6] are ruled out by the following observations. (1) The carotid foramen is too small to have conducted an internal carotid artery of significant size. The diameter of the carotid foramen in *Ignacius* is comparable to that of lorises, in which the internal carotid artery involutes during development and only the internal carotid nerve passes through[15,16]. (2) There are no grooves or tubes for the promontory division of the internal carotid artery on the promontorium. It has been suggested that in *Phenacolemur* the promontorial branch travelled through a longitudinal septum running anteriorly from the promontorium[3]. In *Phenacolemur*, however, this strut lacks a lumen for transmitting an artery[11]. In USNM 421608 the homologous strut is broken rostrally and the broken part likewise shows no lumen. Moreover, the longitudinal septum projects too far laterally for it to transmit an artery to the cerebral arterial circle. (3) There are no grooves on the basioccipital or basisphenoid along the cranial base, nor is there a middle lacerate foramen to transmit an ascending pharyngeal artery from the neck to the inside of the braincase as in cheirogaleids and lorises[15]. Foramina and grooves for these structures previously identified on another more fragmentary

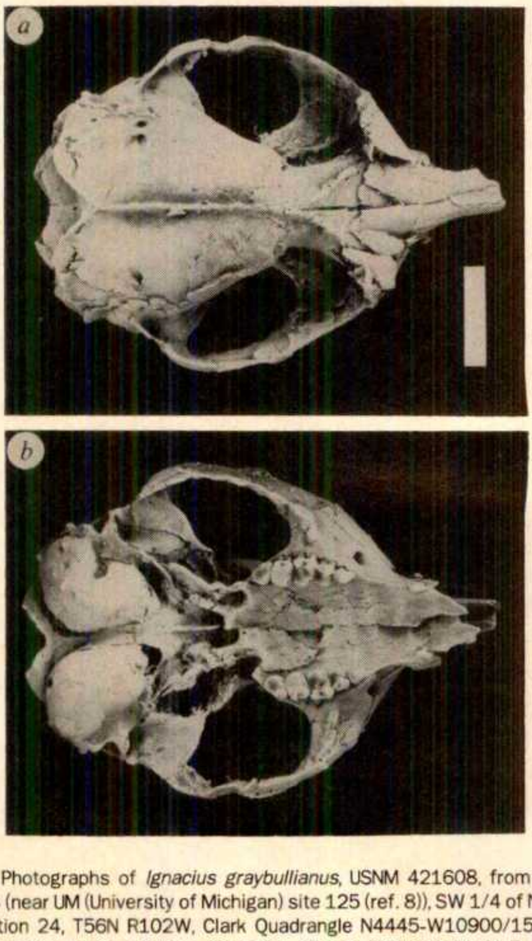

FIG. 1 Photographs of *Ignacius graybullianus*, USNM 421608, from Houde site 24 (near UM (University of Michigan) site 125 (ref. 8)), SW 1/4 of NW 1/4 of section 24, T56N R102W, Clark Quadrangle N4445-W10900/15 1950. *a*, Dorsal view; *b*, ventral view. Scale bar, 10 mm. Photography, V. E. Krantz.

skull of *Ignacius*[6] are perhaps for the inferior petrosal venous sinus that ran inside the braincase or are artefacts of the separation of bones on the cranial base during fossilization[5]. By the process of elimination, the principal intracranial blood supply of *Ignacius* was probably from the vertebral arteries by way of the foramen magnum. This means of supplying intracranial blood, together with the exclusion of an internal carotid artery and of an ascending pharyngeal artery from such a role, is a synapomorphy of *Plesiadapis*[11], *Ignacius* and extant Dermoptera[14,17].

The anatomical features described above provide new insight into the phylogenetic position of Plesiadapiformes. Plesiadapiformes lack convincing craniodental or postcranial synapomorphies with Primates. They do not have such distinctively primate features as a postorbital bar, orbital convergence with narrowed interorbital space, an enlarged brain, a large internal carotid artery in a promontorial position enclosed in a bony canal, or a petrosal auditory bulla. Previously suggested postcranial synapomorphies[5,9] linking Plesiadapiformes with Primates also seem to be unfounded[18-20]. About the only derived similarity between Plesiadapiformes and some early Eocene Primates is the presence of a *Nannopithex*-fold on the upper molars[21]. However, *Nannopithex*-folds are poorly developed in the earliest primates of modern aspect (such as *Cantius* or *Teilhardina*) suggesting that the feature evolved independently in Plesiadapiformes and Primates. In the absence of any convincing evidence linking Plesiadapiformes with primates of modern aspect, there is now no need for the term Euprimates (see ref. 5) as distinct from Primates.

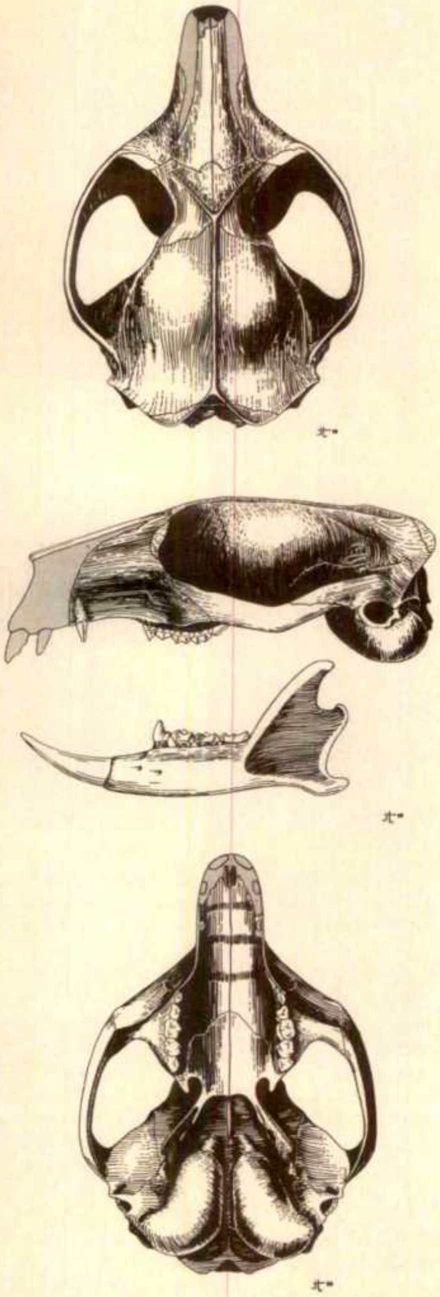

FIG. 2 Reconstruction of *Ignacius* based principally on USNM 421608 and the premaxilla (represented by grey stipple) of a previously described specimen of this species[7]. The lower jaw in the reconstruction is based on an immature specimen from Houde site 14 (probably within UM site SC 26) also from early Wasatchian rocks of the Willwood Formation, Clark's Fork Basin, Wyoming. Drawing, J. Trecartin.

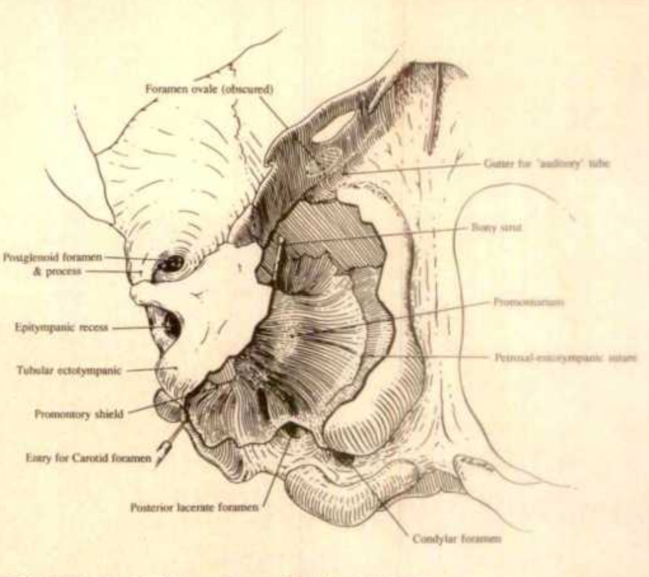

FIG. 3 The right auditory region of USNM 421608 from a ventral perspective. Part of the auditory bulla has been removed. Drawing, B. Smith.

The hypothesis that Plesiadapiformes may form a monophyletic clade with Dermoptera[19,20] is supported by the following cranial synapomorphies. (Recent evidence shows that Eocene Plagiomenidae have no close relationship to Dermoptera as previously supposed[22].) Extant Dermoptera and Plesiadapiformes share the collar-shaped ectotympanic, a partially involuted internal carotid arterial system, a bulla composed partly of the entotympanic, and an entotympanic that contacts the basioccipital medially. The last two features are demonstrated in Plesiadapiformes only for *Ignacius*, but the reported absence of similar sutural patterns in the fully ossified bulla of *Plesiadapis* does not rule out the possibility of an entotympanic in this taxon as well[6,11]. Further, we find no features of the cranial anatomy to support the hypothesis that Primates, tree shrews, bats and dermopterans form a clade Archonta. A hypothetical ancestor of such a group would have resembled primitive eutherians in having an unossified cranial bulla and unreduced internal carotid system. In summary, the new cranial and postcranial fossils of paromomyid Plesiadapiformes discussed here and elsewhere[19,20] indicate that these animals were not 'archaic primates' as commonly believed[1-5,9,21]. Rather, they appear to be more aptly termed 'archaic dermopterans'. ☐

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1. Gingerich, P. D. *Univ. Mich. Pap. Paleont.* **15,** 1-141 (1976).
2. Russell, D. E. *Mem. Mus. nat. Hist.* **13,** 1-324 (1964).
3. Szalay, F. S. *Am. J. phys. Anthrop.* **36,** 59-76 (1972).
4. Szalay, F. S. *Bull. Am. Mus. nat. Hist.* **140,** 193-330 (1969).
5. Szalay, F. S., Rosenberger, A. L. & Dagosto, M. *Yb. phys. Anthrop.* **30,** 75-105 (1987).
6. MacPhee, R. D. E., Cartmill, M. & Gingerich, P. D. *Nature* **301,** 509-511 (1983).
7. Rose, K. D. & Gingerich, P. D. *Contrib. Mus. Paleont. Univ. Mich.* **24,** 181-189 (1976).

8. Gingerich, P. D. *Wyoming Geol. Assn Guidbks* **34,** 185-195 (1983).
9. Szalay, F. S. & Delson, E. *Evolutionary History of the Primates* 1-580 (Academic, New York, 1979).
10. Novacek, M. J. *Mammal. Rev.* **7,** 131-149 (1977).
11. MacPhee, R. D. E. & Cartmill, M. in *Comparative Primate Biology: Systematics, Evolution and Anatomy* (eds Swindler, D. & Erwin, J.) 219-275 (Alan R. Liss, New York, 1986).
12. Novacek, M. J. *Bull. Am. Mus. nat. Hist.* **183,** 1-112 (1986).
13. Szalay, F. S. *J. hum. Evol.* **6,** 3-18 (1977).
14. Hunt, R. M. & Korth, W. W. *J. Morph.* **164,** 167-211 (1980).
15. Cartmill, M. in *Phylogeny of the Primates: A Multidisciplinary Approach* (eds Luckett, W. P. & Szalay, F. S) 313-354 (Plenum, New York, 1975).
16. Conroy, G. C. & Packer, D. J. *Folia primatol.* **35,** 237-247 (1981).
17. Wible, J. R. *Zool. J. Linn. Soc.* **91,** 107-135 (1987).
18. Wible, J. & Covert, H. H. *J. hum. Evol.* **16,** 1-22 (1987).
19. Beard, K. C. thesis, The Johns Hopkins University, Baltimore, MD (1989).
20. Beard, K. C. *Nature* **345,** 340-341 (1990).
21. Hoffstetter, R. *C. r. hebd. Seanc. Acad. Sci., Paris (Ser. II)* **302,** 43-45 (1986).
22. MacPhee, R. D. E., Cartmill, M. & Rose, K. D. *J. vert. Paleont.* **9,** 329-349 (1989).

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Limited heterogeneity of rearranged T-cell receptor Vα transcripts in brains of multiple sclerosis patients

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THE identification of activated T cells in the brain of individuals with multiple sclerosis (MS) indicates that these cells are critical in the pathogenesis of this disease. In an attempt to elucidate the nature of the lymphocytic infiltration, we used the polymerase chain reaction to amplify T-cell antigen receptor (TCR) Vα sequences from transcripts derived from MS brain lesions. In each of three MS brains, only two to four rearranged TCR Vα transcripts were detected. No Vα transcripts could be found in control brains. Sequence analysis of transcripts encoded by the Vα 12.1 region showed rearrangements to a limited number of Jα region segments. These results imply that TCR Vα gene expression in MS brain lesions is restricted.

Multiple sclerosis is an inflammatory disease of the central nervous system (CNS), characterized by myelin destruction[1-3]. In the brain, there is an accumulation of macrophages, plasma cells, major histocompatibility complex (MHC) class II positive antigen-presenting cells and activated cytokine-secreting T cells[4-8]. Several lines of evidence indicate that T lymphocytes migrate from the peripheral blood to the CNS compartment and participate directly in the formation of brain lesions[9-11]. There is also evidence of oligoclonality in T lymphocytes within the cerebrospinal fluid of MS patients[12]. In addition, TCR Vα and Vβ genes have been shown to contribute to the genetic control of susceptibility to this disease[13-15].

To examine the expression of TCR genes at the site of disease, messenger RNA isolated from demyelinating brain plaques from three MS patients with chronic progressive disease, and from three control brains (non-MS) was used to synthesize complementary DNA. These cDNAs were then subjected to enzymatic gene amplification by the polymerase chain reaction (PCR) method[16,17] using specific TCR primers. The results of such an amplification using primers for the TCR Vα 12.1 family

TABLE 1 T-cell receptor α-primers

Primer	Clone	Sequence	Family members
Vα 1	HAP 10	5'-CTGAGGTGCAACTACTCA-3'	1.1, 1.2, 1.3
Vα 2	HAP 26	5'-GTGTTCCCAGAGGGAGCCATTGCC-3'	2.1, 2.2
Vα 3	HAP 05	5'-GGTGAACAGTCAACAGGGAGA-3'	3.1
Vα 4	HAP 08	5'-ACAAGCATTACTGTACTCCTA-3'	4.1
Vα 5	HAP 35	5'-GGCCCTGAACATTCAGGA-3'	5.1
Vα 6	HAP 01	5'-GTCACTTTCTAGCCTGCTGA-3'	6.1
Vα 7	HAP 21	5'-AGGAGCCATTGTCCAGATAAA-3'	7.1, 7.2
Vα 8	HAP 41	5'-GGAGAGAATGTGGAGCAGCATC-3'	8.1, 8.2
Vα 9	HAP 36	5'-ATCTCAGTGCTTGTGATAATA-3'	9.1
Vα 10	HAP 58	5'-ACCCAGCTGGTGGAGCAGAGCCCT-3'	10.1
Vα 11	HAP 02	5'-AGAAAGCAAGGACCAAGTGTT-3'	11.1
Vα 12	PGA 5	5'-CAGAAGGTAACTCAAGCGCAGACT-3'	12.1
Vα 13	AB 11	5'-GCTTATGAGAACACTGCGT-3'	13.1
Vα 14	AB 21	5'-GCAGCTTCCCTTCCAGCAAT-3'	14.1
Vα 15	AC 24	5'-AGAACCTGACTGCCCAGGAA-3'	15.1
Vα 16	AE 212	5'-CATCTCCATGGACTCATATGA-3'	16.1
Vα 17	AF 211	5'-GACTATACTAACAGCATGT-3'	17.1
Vα 18	AC 9	5'-TGTCAGGCAATGACAAGG-3'	18.1
Cα	PGA 5	5'-AATAGGTCGAGACACTTGTCACTGGA-3'	Cα

The size of amplified products using 5' Vα and 3' Cα primers ranged from about 320 to 410 base pairs.

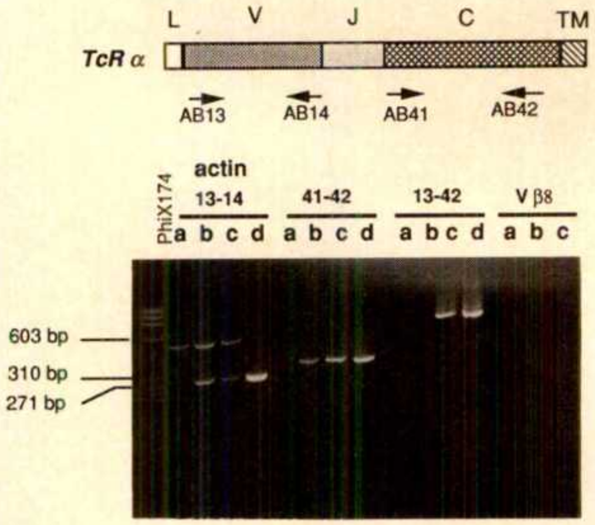

FIG. 1 Brain TCR amplification of MS patient 1. Lanes *a*: control brain cDNA; *b*, MS parietal region brain cDNA; *c*, MS occipital region brain cDNA; *d*, PGA5, a full-length TCR α cDNA[18]. Complementary DNA (2 μl) was combined in a 100-μl reaction volume, with 1 unit of DNA *Taq* polymerase (Perkin Elmer–Cetus), 10 μl 10 × reaction buffer, 50 μM each of deoxynucleoside triphosphates, and 1 μM of each primer. The PCR profile used was: denaturation 95 °C for 60 s, annealing 45 °C for 60 s and extension 72 °C for 60 s, for 35 cycles on a DNA Thermal Cycler (Perkin Elmer–Cetus). One tenth of each sample was independently run in a 4% Nusieve agarose gel (Fmc) and an appropriate size fraction was excised from the gel. The agarose piece was frozen and thawed 3 times, and 2 μl supernatant directly reamplified with the same primers for an additional 25 cycles. A 500-bp actin fragment was successfully amplified from brain cDNA (lanes a–c), but not from the PGA5 control (lane d) using the following primers: 5'-ACGAAGACGGACCACCGCCCTCG-3', 5'-CACGTTGTGGGTGACGCCGTC-3'). Vα and Cα transcripts were amplified from both MS brain cDNA and PGA 5 templates, but not from the control MS brain cDNA with primers AB 13–14 (5'-CAGAAGGTAACTGCAGCGCAGACT-3', 5'-TTGGGGATCCAGAGCACAGAAGTATACTGC-3'), which include *Pst*I and *Bam*HI restriction sites and define a 286-bp fragment of the Vα 12.1 region gene; and AB 41–42 (5'-CAGAACCCTGACCCTGCCGTGTAC-3', 5'-GTGTCCACAGTTTAGGTTCGTATCTGT-3', which includes a *Sal*I site and defines a 340-bp fragment of the Cα region transcript) respectively. Note that rearranged TCRα sequences could be amplified from cDNA of the MS brain prepared from the occipital region (lane *c*) using the Vα 12.1 primer AB 13 and Cα primer AB 42.

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TABLE 2 T-cell receptor Vα expression in brain plaques of multiple sclerosis patients

	Vα 1	Vα 2	Vα 3	Vα 4	Vα 5	Vα 6	Vα 7	Vα 8	Vα 9	Vα 10	Vα 11	Vα 12	Vα 13	Vα 14	Vα 15	Vα 16	Vα 17	Vα 18	Vβ 8	Actin
Experiment no. 1																				
MS Br1	383	4,640	760	520	240	850	826	1,566	450	*45,860*	5,430	*36,380*	3,618	367	280	289	226	442	170	*104,450*
MS Br2	140	824	523	310	830	415	660	*23,200*	1,750	*29,630*	623	*49,125*	456	220	317	*12,460*	3,572	338	280	*79,120*
MS Br3	638	313	276	410	817	1,520	210	*15,860*	*16,310*	*21,200*	838	2,050	302	225	462	3,633	482	470	630	*58,358*
C Br4	235	1,100	135	115	286	7,300	427	960	1,036	317	560	726	485	278	466	630	545	830	900	*65,996*
C Br5	580	875	180	490	110	846	160	324	780	120	344	138	762	755	876	860	715	570	860	*66,393*
C Br6	137	290	133	530	836	640	910	110	140	350	670	1,030	1,095	2,000	437	775	240	330	710	*139,337*
Experiment no. 2																				
MS Br1	1,650	3,956	1,450	790	547	545	1,170	343	1,856	*32,870*	513	*12,978*	866	868	3,190	280	1,048	1,127	440	*38,593*
MS Br2	967	340	1,419	1,575	3,866	2,837	1,848	*13,373*	2,974	*17,337*	1,550	*33,020*	1,487	1,072	3,148	*17,968*	1,446	980	1,338	*32,460*
MS Br3	666	726	1,198	790	1,769	258	576	*35,270*	*18,990*	*19,138*	948	2,690	587	880	815	945	946	1,570	630	*22,415*
C Br4	1,507	660	1,740	1,790	553	706	4,540	4,410	1,333	584	919	765	860	206	590	713	2,748	526	864	*31,285*
C Br5	896	1,670	2,370	5,000	2,826	418	862	8,175	2,048	1,307	1,734	836	737	1,040	2,097	2,925	1,025	5,276	4,478	*33,018*
C Br6	883	1,727	716	865	610	1,334	9,514	1,033	1,256	1,130	636	170	4,636	1,300	1,930	1,167	764	5,915	370	*29,451*
PBL(PHA)	*9,434*	*19,464*	*8,288*	*18,434*	*18,820*	*10,483*	*12,800*	*14,886*	*13,980*	*23,040*	*11,448*	*16,968*	*16,536*	*17,750*	*30,512*	*16,544*	*21,132*	*19,732*	ND	ND

Samples were taken from brain plaques of three patients with chronic progressive MS, and three controls (non-MS). Total RNA and cDNA (from 5 μg RNA) were prepared according to standard procedures[33]. Control cDNA was also prepared from 1 μg RNA isolated from a pool of peripheral blood lymphocytes from five different individuals, stimulated with 3 μg ml^{-1} of PHA. cDNAs were amplified with TCR Vα–Cα or actin primers for 40 cycles in the presence of 10 μCi of α-[^{32}P]dATP (Amersham). Samples were analysed by gel electrophoresis with ethidium bromide to identify the specific fragment band (italic figs when a band was clearly visible). After electrophoretic separation, bands were excised and incorporation of radiolabel was determined. Where TCR rearranged bands were absent, an agarose fragment 200–600 bp was excised and counted. An actin band was visualized in all the amplified brain cDNA. Results are expressed in counts per min. All TCR 5′ primers amplify TCR sequences from germ line DNA using a specific 3′ Vα primer for each family. We have detected V-Cα rearrangements of all TCR V gene members in a variety of activated T cells including single rearrangements of specific Vα members in T-cell clones reactive to pertussis toxin, to Borrelia bergdorfei, as well as the Jurkat T cell line, and rearrangements of all Vα members in pooled T cells stimulated by PHA (line 7, experiment 2). ND, Not determined.

on cDNA isolated from the parietal and occipital brain regions from one MS patient, and from the occipital brain region of one control (non-MS) individual, are shown in Fig. 1. Actin sequences were coamplified together with the Vα 12.1 gene to monitor the integrity of the cDNAs. Actin could be amplified from the brain cDNAs (500 base pair (bp) fragment (actin primer: lanes a–c) but not from PGA5, a full-length cDNA clone that contains the Vα 12.1 segment[18] (lane d). A smaller PCR product corresponding to the Vα 12.1 gene was detected in the patient but not in the control sample (282-bp fragment; primer AB13-AB14: lanes b, c and d). To ensure that only the Vα 12.1 family was amplified, genomic and brain cDNA PCR products were analysed using restriction endonucleases and were consistent with the known restriction map for Vα 12.1. When colonies containing cloned Vα PCR products from MS brain cDNA were screened with a Vα 12.1 region probe, about 20% were positive. DNA from several of these colonies was sequenced, and was found to be identical with the Vα 12.1 sequence[18]. Thus, the TCR Vα 12.1 restriction fragment-length polymorphism recently associated with MS susceptibility[15], must be in a sequence flanking the Vα 12.1 gene. Specific regulation of the α-TCR gene by 3′ *cis*-acting enhancers, was recently demonstrated[19].

These experiments indicated that PCR could amplify the receptor transcripts from post-mortem brain samples, starting from nanograms of total RNA without the necessity of *in vitro* expansion of T cells. Similarly, Cα sequences were amplified from MS brain cDNAs, but not from the control sample (primers AB41-AB42: lanes b and c, Fig. 1). A subsequent amplification was carried out (primers AB13-AB42: lanes a, b, c and d) using a 5′ primer complementary to the Vα 12.1 and a 3′ primer complementary to the Cα TCR regions. These primers, which can amplify only rearranged TCR transcripts, amplified a product of ~680 bp from both the positive control PGA5 sample and cDNA from the occipital region of the MS brain (primers AB13-AB42: lanes c and d) but not from control brain cDNA or cDNA from the parietal region of the MS brain (primers AB13-AB42: lanes a and b). The Vα and Cα amplifications from the MS brain parietal region most probably represent transcripts from unrearranged chromosomes, as has been found in other cDNA libraries from T cell lines[20]. No PCR product was observed using primers corresponding to the Vβ 8 family, even though this TCR Vβ region was recently reported to be associated with susceptibility to MS (ref. 13).

To substantiate that the DNA produced by PCR was an authentic amplified product of rearranged TCR genes, the PCR products were sequenced using the dideoxy chain termination method[21] after double screening of colonies with Vα and Cα probes. Only two different J regions were seen in the 25 clones examined. Both were different from the PGA5 Jα sequence, ruling out the possibility of a 'carryover' contamination. Eleven clones contained the JαO family found in clone HAP 41 (ref.

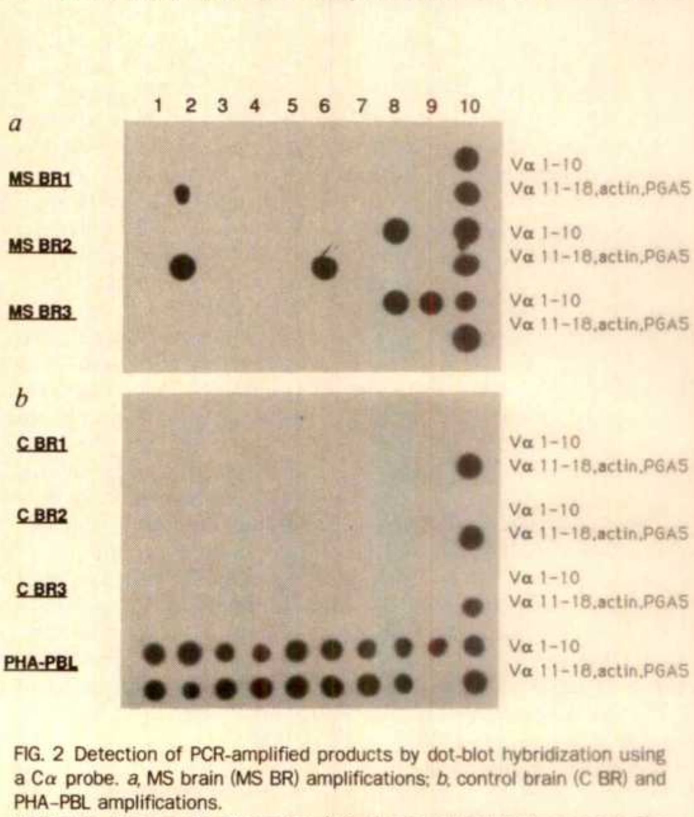

FIG. 2 Detection of PCR-amplified products by dot-blot hybridization using a Cα probe. *a*, MS brain (MS BR) amplifications; *b*, control brain (C BR) and PHA–PBL amplifications.

METHODS. The amplified product (10 μl) was dot-blotted onto transfer membranes (GeneScreen Plus, NEN) after denaturation with 0.4 M NaOH–25 mM Na$_2$EDTA. Actin PCR products served as negative controls and PGA5 as the positive control. Filters were fixed under ultraviolet light and prehybridized for 3 h at 42 °C in 5×SSPE/5× Denhardt's solution/salmon sperm DNA (100 μg ml^{-1})/0.1% SDS and hybridized overnight at 42 °C with 1×10^6 c.p.m. per ml of ^{32}P-kinased probe (5′-AATATCCAGAACCCTGACCCT-3′). Filters were washed in 1×SSPE/0.1% SDS at room temperature, twice for 20 min; and then in 0.1×SSPE/0.1% SDS at 45 °C twice for 10 min. Kodak XAR-5 film with Dupont Lightning-Plus intensity screens were used for autoradiography at −70 °C for 30 min. Signals correlate with the presence of a specific band in the ethidium bromide-stained agarose gel and with high [^{32}P]dATP incorporation during amplification (Table 2).

22) and 14 clones had a previously undescribed Jα sequence (GGGTACCGAGATGACGAACCCACCTTTGGGACAGGCACTCAGCTAAAAGTGGAACTC).

We next asked whether there was a diverse or limited usage of TCR Vα gene expression in MS brain lesions. To analyse the use of TCR Vα in MS brains, we synthesized 5′ PCR primers for the 18 different Vα families[22,23] (Table 1). Optimal conditions for amplification were ascertained using each of the 5′ primers (Table 1) in combination with a specific 3′ Vα primer for each TCR Vα family, on genomic DNA (data not shown) and with a common Cα primer (AB 51) on reverse-transcribed RNA isolated from phytohaemagglutunin (PHA)-stimulated peripheral blood lymphocytes (Table 2). The results from amplification of MS brain cDNA, using 5′ Vα primers and the common 3′ Cα primer AB 51 in the presence of α-[^{32}P]dATP, showed that in each brain only a few TCR Vα gene families are preferentially rearranged and transcribed (Table 2). Visualization of a specific band for actin or rearranged TCR after gel electrophoresis of the amplified product, is indicated by italics in Table 2. In general, high [^{32}P]dATP incorporation was reproducibly detected in regions of the gel where bands were visualized.

These results were further confirmed by dot blot of the PCR products and hybridization with a radiolabelled oligonucleotide corresponding to a TCR-Cα sequence, 5′ from the common Cα primer (Fig. 2). A clear signal in the dot-blot assay correlates with the presence of a band staining with ethidium bromide in the agarose gel. Products of amplifications with actin primers were used as a negative control; amplification products of clone PGA5 with TCR Vα and Cα primers were used as a positive control. Expression of TCR Vα transcripts is operationally defined by (1) the presence of a rearranged Vα–Cα band visualized with ethidium bromide, and (2) a signal on dot-blot hybridization of the amplified Vα–Cα gene with a TCR Cα probe, 5′ from the Cα PCR primer. Thus in MS brain 1, TCR Vα 10 and 12 were rearranged; TCR Vα 8, 10, 12 and 16 were rearranged in MS brain 2, and Vα 8, 9 and 10 were rearranged in MS brain 3. No Vα transcripts could be amplified from control brain cDNA, although actin could be amplified. Each of 18 TCR Vα rearranged genes were amplified from PHA-stimulated pooled lymphocytes. The Vα 10 family was detected in all three MS samples, suggesting that this TCR might be responding to a major epitope of an antigen involved in the pathogenesis of MS. Further experiments will be needed to confirm this hypothesis.

Our results may prove to have therapeutic implications. Studies on TCR gene expression in experimental allergic encephalomyelitis (EAE), a prototypic animal model for autoimmunity induced by T cells, have shown the predominant use of certain TCR α and β products in the immune response to myelin basic protein[24–27]. Treatment of mice *in vivo* who have EAE with monoclonal antibody specific to the predominant TCR V-region product reverses paralytic disease[24]. In addition, it is possible to prevent EAE by immunization with inactive encephalitogenic T cells[28] or with synthetic peptides from the CDR2 and CDR3 TCR V regions that are rearranged in the encephalitogenic clones[29,30]. Elucidation of TCR expression in the brain may help in the design of similar treatments in MS patients. ☐

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1. McFarlin, D. E. & McFarland, H. F. *New Engl. J. Med.* **307,** 1183–1251 (1982).
2. Raine, C. S. & Traugott, U. in *Immunoregulatory Processes in Experimental Allergic Encephalomyelitis and Multiple Sclerosis* (eds Alvord, E. A. & Kies, M. W.) 151–212 (Elsevier, New York, 1984).
3. Prineas, J. W. & Wright, R. G. *Lab. Invest.* **38,** 409–421 (1978).
4. Prineas, J. W. *Handbook of Clinical Neurology,* Vol. 3, 213–257 (Elsevier, New York, 1985).
5. Woodroof, M. N. *et al. J. neurol. Sci.* **74,** 135–152 (1986).
6. Hafler, D. A. & Weiner, H. L. *Ann. Neurol.* **22,** 89–93 (1987).
7. Hafler, D. A. & Weiner, H. L. *Immunol. Rev.* **100,** 307–332 (1987).
8. Hoffman, F. M., Hinton, D. R., Johnson, K. & Merril, J. E. *J. exp. Med.* **170,** 607–612 (1989).
9. Hoffman, F. M. *et al. J. Immun.* **136,** 3239–3245 (1986).
10. Traugott, U., Reinherz, E. L. & Raine, C. S. *J. Neuroimmun.* **4,** 201–221 (1983).
11. Sobel, R. A. *et al. J. Immun.* **140,** 2210–2214 (1988).
12. Hafler, D. A. *et al. J. exp. Med.* **167,** 1313–1322 (1988).
13. Beall, S. S. *et al. J. Neuroimmun.* **21,** 59–66 (1989).
14. Seboun, E. *et al. Cell* **57,** 1095–1100 (1989).
15. Oksenberg, J. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 988–992 (1989).
16. Saiki, R. K. *et al. Science* **230,** 1350–1354 (1985).
17. Mullis, K. B. & Faloona, F. *Meth. Enzym.* **155,** 335–350 (1987).
18. Sim, G. K. *et al. Nature* **312,** 771–775 (1984).
19. Winoto, A. & Baltimore, D. *EMBO J.* **8,** 729–733 (1989).
20. Loh, E. Y., Elliot, J. F., Cwirla, S., Lainer, L. L. & Davis, M. M. *Science* **243,** 217–220 (1989).
21. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
22. Yoshikai, Y., Kimura, N., Toyonaga, B. & Mak, T. W. *J. exp. Med.* **164,** 90–103 (1986).
23. Klein, M. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 6884–6888 (1987).
24. Acha-Orbea, H. *et al. Cell* **54,** 263–2173 (1988).
25. Urban, J. L. *et al. Cell* **54,** 577–582 (1988).
26. Sakai, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 8608–8612 (1988).
27. Heber Katz, E. & Acha-Orbea, H. *Immunol. Today* **10,** 164–169 (1989).
28. Ben-Nun, A., Wekerle, H. & Cohen, I. R. *Nature* **292,** 60–61 (1981).
29. Vandenbark, A. A., Hashim, G. & Offner, H. *Nature* **341,** 541–544 (1989).
30. Howell, M. D. *et al. Science* **246,** 668–670 (1989).

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Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter

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INHIBITORY non-adrenergic non-cholinergic (NANC) nerves are thought to be important in the autonomic innervation of the gastrointestinal tract and other organ systems. The nature of their neurotransmitter is still debated. Speculation that nitric oxide (NO), formed from L-arginine in neuronal structures[1] and other cells[2], could act as a neurotransmitter, is not yet supported by demonstration of its release upon nerve stimulation. Using a superfusion bioassay, we report the release of a vasorelaxant factor upon stimulation of the NANC nerves[3] in the canine ileocolonic junction. Several pieces of evidence, including the selectivity of the bioassay tissues, chemical instability, inactivation by superoxide anion and haemoglobin, inhibition by N^G-nitro-L-arginine (L-NNA)[4] and potentiation by L-arginine all indicated that NO accounted for the biological activity of this transferable NANC factor.

The canine ileocolonic junction (ICJ) was isolated, superfused with Krebs-Ringer solution and the effluent superfused de-endothelialized rings of rabbit aorta[5], arranged either in a cascade[6,7] (Fig. 1) or in parallel. Upon electrical stimulation (ES; 16 Hz, 2 ms) or infusion of the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP), ICJ tissue released a vasodilator activity, causing 22.4 ± 3.2% ($n = 12$) and 26.3 ± 3.9% ($n = 9$) relaxation of the top tissue respectively. The release of the factor was frequency-dependent and its activity declined (39 ± 8%, $n = 8$, ES; 41 ± 15%, $n = 4$, DMPP) during passage down the cascade. As atropine and guanethidine were present and as the detector tissues failed to relax to acetylcholine (ACh) and noradrenaline (Figs 1 and 2), the transferable factor is indeed non-adrenergic and non-cholinergic. Furthermore, blockade of nerve conductance with tetrodotoxin abolished the release of the vasodepressant factor to both stimuli (Fig. 2), indicating that neuronal structures were activated. Non-selective stimulation of other cell populations[2] or microorganisms therefore seems unlikely.

The instability of the vasodilator factor was compatible with NO[7,8], as confirmed by the injection of authentic NO (Fig. 1). Moreover, haemoglobin (Hb), known to trap NO avidly[7–9], eliminated the biological activity, whereas dilatation, due to NO

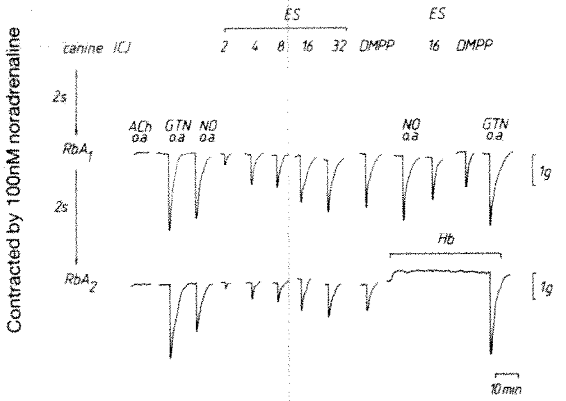

FIG. 1 The ICJ releases an unstable vasodilator in response to ES (2–32 Hz) or infusion of DMPP (30 μM, 60 s, *n* = 4). Biological activity of transferable factor and NO (0.05 nmol, injected over the aortae, o.a.) decreased to the same extent during the delay of 2 s between upper and lower aortic ring. Hb (100 nM) abolished both biological activities instantaneously, without affecting GTN (0.1 nmol, o.a.) responses. After removal of mucosa and submucosa, the ICJ tissue was placed in a superfusion chamber with platinum side walls, connected to a Grass stimulator (square pulses, 2 ms duration, 25 mA, 8 V for 20 s). The luminal side was superfused with Krebs-Ringer buffer (3 ml min^{-1})[5] containing SOD (20 units ml^{-1}), L-arginine (50 μM) and guanethidine (3 μM). The effluent superfused a cascade of two de-endothelialized rabbit aortic rings (RbA) contracted submaximally by 100 nM noradrenaline[5]. ACh (3 nmol, o.a.) injection confirmed the absence of endothelium. Thereafter atropine (300 nM) was introduced into the perfusate and the sensitivity of the bioassay was standardized by injection of GTN. Hb and NO solutions were prepared as described[9].

formed from nitroglycerin (GTN) within the assay tissues, still occurred. Further evidence for the release of NO is provided by the findings that L-arginine, the precursor[1,2] of NO, and superoxide dismutase (SOD), which opposes its inactivation by superoxide anion[6,10], enhanced the vasodepressant activity released from the ICJ (Fig. 2). The relaxations of the top aortic ring were augmented from 8.2 ± 4.2% to 24.2 ± 4.4% (*n* = 5) by SOD. Finally, L-NNA, an inhibitor of NO biosynthesis[4] inhibited the release of the factor (Fig. 2).

Our data clearly demonstrate the release of NO during nerve stimulation. A role for NO in neurotransmission had already been postulated, and neuronal structures have the capacity to generate NO[1,11]. Moreover, NO mimics NANC relaxations, blockers of NO biosynthesis inhibit these relaxations and L-arginine reverses this inhibition in the rat anococcygeus muscle[12,13] and in the canine ICJ (G.E.B. *et al.*, submitted for publication). Our finding that NO is released upon nerve stimulation adds further evidence to propose NO or an NO-releasing molecule as inhibitory NANC neurotransmitter and extends the presence of the L-arginine:NO pathway[2] to gastrointestinal tissue. □

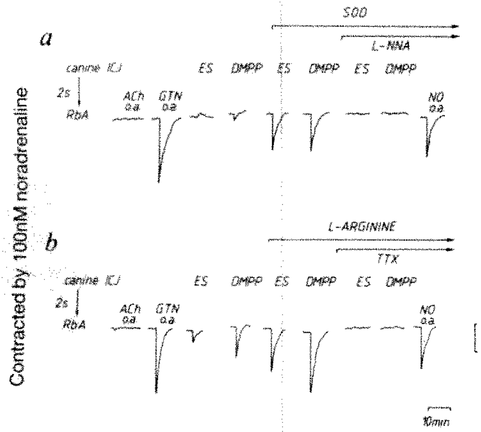

FIG. 2 Evidence for release of NO and its neuronal origin. SOD (20 U ml^{-1}, *a*) and L-arginine (50 μM, *b*) enhance, whereas L-NNA (100 μM, *a*) and tetrodotoxin (TTX, 1 μM, *b*) abolish the release of the vasorelaxant factor in response to ES (16 Hz, 2 ms) and DMPP (30 μM, 60 s). Relaxations induced by NO (0.05 nmol, o.a.) were not prevented. Effluent of two ICJ preparations (*a* and *b*) superfused separate aortic rings. L-Arginine was continuously present in (*a*), and SOD in (*b*). Contraction of assay tissues, confirmation of the absence of endothelium (ACh, 3 nmol, o.a.), atropine addition and calibration (GTN, 0.1 nmol, o.a.) were performed as described for Fig. 1.

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1. Knowles, R. G., Palacios, M., Palmer, R. M. J. & Moncada, S. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5159–5162 (1989).
2. Moncada, S., Palmer, R. M. J. & Higgs, E. A. *Biochem. Pharmac.* **38,** 1709–1715 (1989).
3. Pelckmans, P. A., Boeckxstaens, G. E., Van Maercke, Y. M., Herman, A. G. & Verbeuren, T. J. *Eur. J. Pharmac.* **170,** 235–242 (1989).
4. Mülsch, A. & Busse, R. *Naunyn-Schmiedebergs Archs. Pharmac.* **341,** 143–147 (1990).
5. Bult, H., Fret, H. R. L., Van den Bossche, R. M. & Herman, A. G. *Br. J. Pharmac.* **95,** 1308–1314 (1988).
6. Gryglewski, R. J., Palmer, R. M. J. & Moncada, S. *Nature* **320,** 454–456 (1986).
7. Palmer, R. M. J., Ferrige, A. G. & Moncada, S. *Nature* **327,** 524–526 (1987).
8. Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. & Chaudhuri, G. *Proc. natn. Acad. Sci. U.S.A.* **84,** 9265–9269 (1987).
9. Kelm, M. *et al. Biochem. biophys. Res. Commun.* **154,** 236–244 (1988).
10. Rubanyi, G. M. & Vanhoutte, P. M. *Am. J. Physiol.* **250,** H822–H827 (1986).
11. Garthwaite, J., Charles, S. L. & Chess-Williams, R. *Nature* **336,** 385–388 (1988).
12. Gillespie, J. S., Liu, X. & Martin, W. *Br. J. Pharmac.* **98,** 1080–1082 (1989).
13. Ramagopal, M. V. & Leighton, H. J. *Eur. J. Pharmac.* **174,** 297–299 (1989).

Proton inhibition of *N*-methyl-D-aspartate receptors in cerebellar neurons

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MAMMALIAN neurons contain at least three types of excitatory amino-acid receptors, selectively activated by *N*-methyl-D-aspartate (NMDA) or aspartate, (S)-α-amino-3-hydroxy-5-methyl-4-isoxazole proprionate ((S)-AMPA) and kainate[1]. An important aspect of NMDA receptors is their regulation by a variety of factors such as glycine[2], Mg^{2+} (refs. 3, 4) and Zn^{2+} (refs 5, 6) that are present *in vivo*. We show here that NMDA receptor responses are selectively inhibited by protons, with a 50% inhibitory concentration (IC_{50}) that is close to physiological pH, implying that NMDA receptors are not fully active under normal conditions. (S)-AMPA and kainate responses remain unchanged at similar pH levels. Proton inhibition is voltage-insensitive and does not result either from fast channel block, a change in channel conductance, or an increase in the 50% excitatory concentration (EC_{50}) of aspartate/NMDA or glycine. Instead, protons seem to decrease markedly the opening frequency of 30–50 pS NMDA channels, and reduce the relative proportion of longer bursts. This feature of NMDA receptors could be relevant to neurotoxic activation of NMDA receptors during ischaemia[7], as well as to seizure generation, as extracellular proton changes occur during both of these pathological situations[8,9]. Furthermore, these results may have implications for normal NMDA receptor function as transient changes in extracellular protons occur during synaptic transmission[8,10].

Whole-cell currents produced by bath application of aspartate or NMDA (Fig. 1*a*) were reversibly decreased by 67.3 ± 2.0% (±s.e.m.; *n* = 56) when bath pH was reduced from 7.6 to 6.8; fast-membrane currents produced by brief ionophoretic pulses of aspartate (Fig. 2*a*) were similarly decreased by 69.3 ± 5.3% ($n = 23$ cells). Alternatively, increasing the pH from 7.6 to 8.4 potentiated NMDA receptor currents by 55.3 ± 6.8% ($n = 39$). The calculated IC_{50} for the full inhibition curve (Fig. 1*b*) was pH 7.3, close to physiological pH and also similar to the pK_a values for free cysteine or histidine, suggesting that ionization of these amino-acid residues within the receptor might influence its function. In contrast, kainate and AMPA receptor-mediated currents were insensitive to protons over this pH range, but were inhibited by higher concentrations of H^+, apparently in a non-competitive way (n = 17–32 cells). We estimate that the IC_{50} for (S)-AMPA and kainate is pH 6.3 and 5.7, respectively (Fig. 1*b*). Several reports indicate that H^+ interacts with glutamate receptors[14–17] (L. Vyklicky, V. Vlachova, and J. Krusek, personal communication).

There are many possible sites on the NMDA receptor at which protons might act. These include the binding sites for the agonists NMDA or aspartate, the strychnine-insensitive glycine-binding site[2], the Mg^{2+}-binding site[3,4] and other sites within the channel. We have considered each of these possibilities in turn. Normalized whole-cell dose-response curves constructed at pH 6.8 or 7.6 for aspartate in the presence of 2–3 μM glycine, and also for glycine in the presence of 50 μM aspartate, are shown in Fig. 1*c*. There are no detectable differences in the EC_{50} calculated from the normalized curves for aspartate at pH 6.8 and 7.6, suggesting that proton inhibition is non-competitive with respect to the aspartate-binding site. Decreasing the pH from 7.6 to 6.8 increased the EC_{50} for glycine by 48.4%; however, the clear plateau of the dose-response curve in pH 6.8, and the inability of supplemental glycine (1 mM; $n = 6$ cells) to alter proton inhibition significantly, suggests that the effect of H^+ is non-competitive with respect to glycine. The suggestion[16] that the increase in the estimated K_d for glycine at very low pH (6.3) contributes to proton inhibition through altered gating is consistent with the small shift we observed in the EC_{50}, but our data do not support competitive antagonism of glycine by H^+ over a more physiological pH range (6.8–7.6) in cerebellar granule cells.

Low concentrations of Mg^{2+} are known to block the NMDA receptor channel in a voltage-dependent manner[3,4]. To test whether proton inhibition of NMDA receptor function is also voltage-sensitive, whole-cell currents were recorded in response to aspartate applied ionophoretically over a range of holding potentials at pH 6.8–8.4 (n = 5–6 cells). As illustrated in Fig. 2*b*, aspartate currents reversed close to 0 mV and were linearly related to voltage between −80 to +50 mV in this pH range. The absence of additional rectification in the current-voltage plots at low pH suggests that proton inhibition does not involve a voltage-sensitive mechanism.

Because protons can influence cellular functions in many ways, we examined single-channel currents in various patch configurations[18] to determine whether protons act directly on

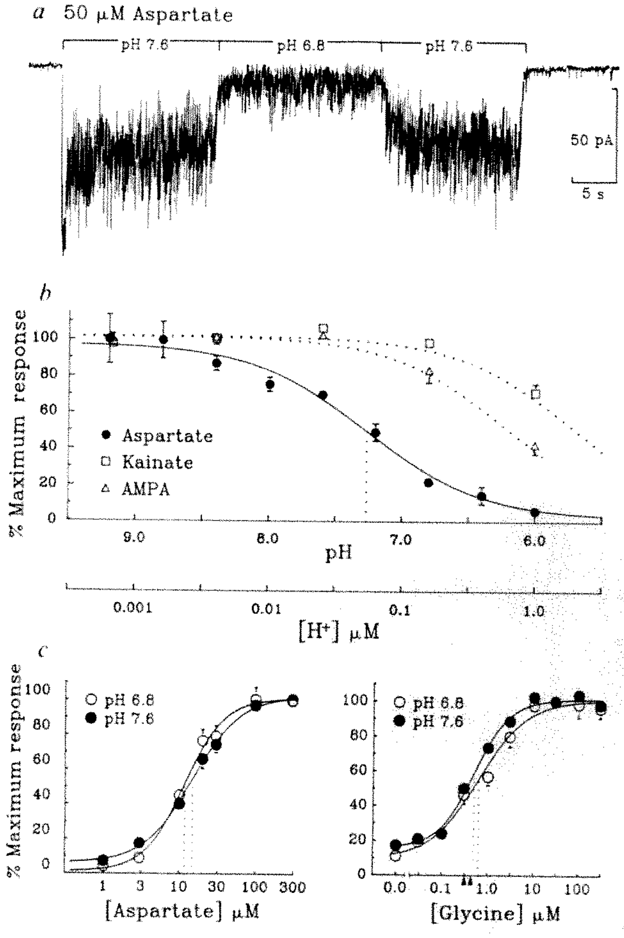

FIG 1 *a*, The influence of protons on the whole-cell response to 50 μM aspartate plus 3 μM glycine applied by perfusion. During application, pH was stepped from 7.6 to 6.8, and back to 7.6 with no change in aspartate or glycine concentrations. *b*, Composite H^+ inhibition curve for NMDA receptor-mediated currents in response to 20–50 μM aspartate plus 3 μM glycine (filled circles). Values were calculated as a percentage of current at pH 7.6, and are shown normalized to 9.2 (±s.e.m. when larger than symbol); measurements were taken from 3–33 cells at each pH. The data were fitted to the equation: $[(\text{maximum}-\text{minimum})/(1+([H^+]/IC_{50}))]+\text{minimum}$. The calculated IC_{50} (dotted line) for aspartate was pH 7.3 (50 nM H^+). Whole-cell currents to 30–50 μM kainate (n = 4–25 cells; open squares) and 10–15 μM (S)-AMPA (n = 4–11 cells; open triangles) at pH 9.2, 8.4, 7.6, 6.8, and 6.0 are included. Aspartate (p$Ka_{-NH_2} = 9.7$) concentrations were adjusted appropriately at pH 8.8 and 9.2, assuming that only the fully ionized species is active. *c*, Dose-response curves for asparate and glycine at pH 6.8 and 7.6 were normalized to the maximum response, and fitted to the equation $[(\text{maximum}-\text{minimum})/(1+(EC_{50}/[\text{aspartate}])^n)]+\text{minimum}$, where n is the Hill coefficient; on average, it was 1.52 for aspartate and 0.70 for glycine. The calculated EC_{50} values to varied aspartate concentration plus 3 μM glycine at pH 6.8 (n = 3–10 cells) and 7.6 (n = 3–12 cells) were 12 and 14 μM, respectively (dotted lines); responses to variable glycine plus 50 μM aspartate had EC_{50} values (arrows) of 0.46 μM in pH 6.8 (n = 4–11 cells) and 0.31 μM in pH 7.6 (n = 4–11) when the minimum was constrained to 0%[26]; dotted lines indicate EC_{50} values calculated with a variable minimum. Values were expressed as a percentage of the mean of control and recovery, and averaged throughout the experiment to compensate for receptor rundown[27].

METHODS. Cerebellar granule cell explants were prepared as previously described[20]. Recordings were made at 22–25 °C from identified granule cells[20]. The bathing solution contained (mM):150 NaCl; 2.8 KCl; 1.0 $CaCl_2$; 10.0 sodium HEPES. If drugs were applied at different pH, the cell was first washed at the new pH to inactivate the transient proton-activated Na^+ current[28]. Our reductions in pH do not significantly alter the ionization of free aspartate or glycine. For whole-cell and outside-out experiments the pipette solution (intracellular) contained (mM):140 CsCl (or 110 CsF, 30 CsCl); 4 NaCl; 5 potassiums EGTA; 5 HEPES; 0.5 $CaCl_2$ pH 7.2; Pipette solutions for cell-attached and inside-out patch experiments consisted of (mM):150 NaCl; 2.8 KCl; 1.0 $CaCl_2$; 10 HEPES plus 30–50 μM aspartate, 3–5 μM glycine at pH 7.2–7.6. Open and shut durations were measured by the time-course fitting method[29]; Bursts were distinguished using a t_{crit} (mean 1.2 ms) calculated between the two brief ($\tau_1 = 67$, $\tau_2 = 537$ μs) and two longer ($\tau_3 = 23.4$, $\tau_4 = 524$ ms; $n = 10$ patches) exponential components describing shut time distributions at pH 7.6. Open probability was calculated from patches subjected to both pH 6.8 and 7.6 using fitted single openings when double openings were <0.5% of total, otherwise determined from the integrated records. The open probability in patches used for burst analysis was, on average, 0.0126 and 0.0039 at pH 7.6 and 6.8, respectively ($n = 8$); fitted parameters were similar in patches with a high *P*(open) compared with low *P*(open). In cell-attached patches, the major aspartate-activated conductance levels were determined to be 40–50 pS; frequent transitions were observed between the 40 and 50 pS levels.

the NMDA receptor-channel complex or, alternatively, through an indirect mechanism. In outside-out patches, where most intracellular constituents are assumed to have been removed, the probability of an aspartate-activated channel (30, 40, 50 pS; glycine present) being open was decreased by 63.6 ± 6.2% when the pH was reduced from 7.6 to 6.8 ($n = 9$ patches; Fig. 2*d*). Furthermore, when cells were subjected to a reduced pH that would block the whole-cell response to aspartate (pH 6.8), single-channel openings produced by apartate in the pipette could still be recorded in cell-attached patches, and the probability that they were open was not significantly different from that in pH 7.6 ($n = 7$ patches; paired *t*-test; power to detect a 60% change was 0.75). Similarly, the probability of aspartate-activated channels being open was not significantly different when the intracellular surface of inside-out patches was exposed to pH 6.8 or 7.6 ($n = 5$ patches; power to detect a 40% change was 0.75). These experiments provide strong support for the idea that H^+ acts at a specific site localized on or near the extracellular face of the NMDA receptor-channel complex.

Although the voltage insensitivity of H^+ inhibition meant that a channel-blocking mechanism was unlikely, it was possible that H^+ produced an incomplete blockage of channel currents through actions at a site outside the membrane electric field, as reported for proton block of Ca^{2+} channels[19]. This could give rise to discrete lower conductance levels at reduced as well as normal pH values. We therefore examined the effect of H^+ on both aspartate-activated single-channel currents in outside-out patches and aspartate or NMDA-induced whole-cell noise. The single-channel conductance estimated from aspartate and NMDA noise spectra fitted with two Lorentzian components[20] was unchanged at both inhibiting and potentiating pH (53 pS at pH 7.6; $n = 33$ spectra in 11 cells; single-factor analysis of variance). Consistent with this result, single-channel conductance levels of approximately 11, 19, 28, 43 or 53 pS (refs 21, 22) were all present in outside-out patches exposed to aspartate at pH 6.8 (Fig. 2*c*), and the relative frequencies of single-channel openings to the 19, 28, 43, 53 pS (refs 20, 23) conductance levels were similar to their counterparts determined at pH 7.6 ($n = 10$ patches).

Analysis of open times (50–85 μs resolution) revealed a decrease of 54.8 ± 10.9% ($n = 10$ patches) for the burst frequency (corrected for missed events). The decreased burst frequency did not appear to reflect enhanced desensitization, as the ratio of peak to steady-state somatic currents was insensitive to the pH (glycine present; $n = 10$ cells). The mean duration of all bursts was also decreased by 38.2 ± 7.7% ($n = 10$). Taken together, these measured decreases in burst frequency and mean burst length at pH 6.8 would be expected to cause a 66.1% reduction in whole-cell current, and therefore would fully account for our whole-cell measurements. To examine the effect of H^+ on burst characteristics, we compared the three fitted components[20] that described distributions both of burst lengths and of individual open times at pH 7.6 and 6.8. Protons did not significantly change the fitted time constants describing either

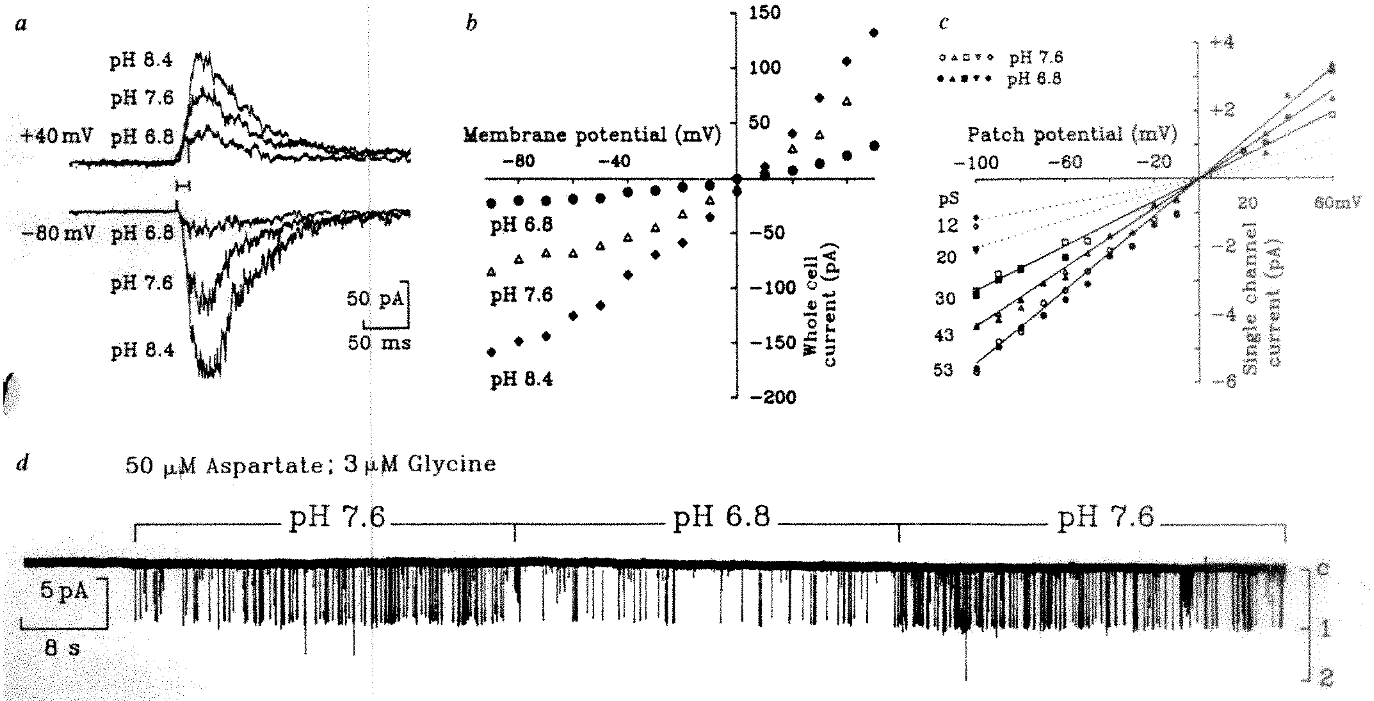

FIG. 2 *a*, Whole-cell responses to brief (10–30 ms) ionophoretically applied aspartate (indicated by bar; 100–250 nA; pipette resistance 90–130 MΩ) in a cell at +40 and −80 mV bathed in pH 6.8, 7.6, and 8.4 (3 μM glycine in the bath). Whole-cell currents were digitized at 1 kHz (filtered 0.5 kHz, −3dB; calibration 50 pA, 50 ms), averaged (3–5), and 100 ms of the peak current measured. *b*, Whole-cell current-voltage relationship in response to ionophoretically applied aspartate in nominally Mg^2-free solution (plus 3 μM glycine) at pH 6.8, 7.6, 8.4. *c*, Single-channel current-voltage relationship for multiple conductance levels produced by aspartate at pH 6.8 ($n = 4$) and 7.6 ($n = 5$) over the range −100 to +80 mV. Current amplitudes were extracted from amplitude histograms constructed from the digitized data selected for channel open times; histograms were fitted to the sum of 1–5 Gaussian densities[21]; 50–1000 openings were analysed at each potential. Low-variance mean amplitude histograms[30] were constructed to aid in sublevel detection. Mean current amplitudes for the ~12, 20 pS conductance levels are only shown at −100 mV for clarity. Slope conductances calculated (linear regression) from the averaged currents at pH 7.6 were 53.0, 42.5, 31.6, 21.1, 12.2 pS; and at pH 6.8 were 53.4, 42.6, 27.6, 19.2, 11.4 pS. Lines are for averaged slopes at pH 7.6 and 6.8, constrained through the origin. The average relative opening frequencies ($n = 10$ patches) for the multiple conductance levels at −80 mV in pH 6.8, 7.6 were 0.65, 0.76 (53 pS); 0.19, 0.15 (43 pS); 0.03, 0.03 (30 pS); 0.04, 0.03 (20 pS); 0.09, 0.03 (12 pS). *d*, Aspartate-induced single-channel currents in an outside-out patch at −80 mV, recorded on slow time-base; calibration 5 pA, 8 s. 50 μM aspartate plus 3 μM glycine was applied externally at pH 7.6, and the pH stepped to 6.8 without a change in agonist concentration, and back to 7.6 (as indicated). The probability of being open for this patch was 0.0121 in pH 7.6, and 0.0037 in pH 6.8. The closed level (c) and amplitude of 1, 2 openings are indicated. These data were filtered at 2.5 kHz (−3dB).

of these distributions, for which the burst lengths at pH 7.6 were 0.05 ± 0.01, 1.21 ± 0.33, and 6.13 ± 0.51 ms ($n = 8$ patches). Instead, the decrease in overall mean burst length in patches at pH 7.6 and subsequently at pH 6.8 reflected a decrease of $42.9 \pm 9.9\%$ ($n = 7$ patches; $P < 0.05$; paired t-test) in the relative fitted area of the longest component. Thus, although the time constants describing burst distributions did not change, there seemed to be a smaller proportion of long bursts. Analogous results were found in individual open time histograms (data not shown).

Substantial decreases in extracellular pH (~0.6 units[10,24]) are known to accompany increased extracellular glutamate levels[7] during experimental ischaemia, and they might attenuate neurotoxic activation of NMDA receptors[7]. This possibility is supported by findings that reduced pH protects cultured neurons from glutamate-induced and hypoxia-induced toxicity[25]. Furthermore, the activation of synaptic NMDA receptors contributes to seizure generation in most experimental situations[7], and the biphasic changes in extracellular H^+ reported for stimulus-induced seizures *in vivo*[9] could influence synaptic NMDA receptors, and therefore seizure time course. Indeed, this idea is consistent with recent findings that small changes in H^+ (±0.1 pH unit) alter neuronal excitability and modulate seizure threshold[9].

The proton sensitivity of NMDA receptors could also have important implications for normal neuronal function. Extracellular pH is known to change by up to 0.4 pH units during electrical stimulation of cerebellar or hippocampal slices[8,11], or of cerebellar parallel fibres *in vivo*[10]. Moreover, from our results, acidic changes of such magnitude should inhibit NMDA receptors by $45.9 \pm 9.2\%$ ($n = 7$ cells). Of particular interest is the possibility that the fast (~10 ms) acid transient previously reported[11] might arise from acidification of the cleft by the contents of synaptic vesicles, as an H^+-ATPase provides the electrochemical H^+ gradient (inside is acidic and positively charged) that drives vesicular glutamate uptake[12,13]. This would inhibit NMDA receptors, but not kainate or AMPA receptors, and add another level of complexity to synaptic events. □

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1. Watkins, J. C. & Evans, R. H. *A. Rev. Pharmacol. Toxicol.* **21,** 165–204 (1981).
2. Johnson, J. W. & Ascher, P. *Nature* **325,** 529–531 (1987).
3. Mayer, M. L., Westbrook, G.L. & Guthrie, P.B. *Nature* **309,** 261–263 (1984).
4. Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307,** 462–465 (1984).
5. Peters, S., Koh, J. & Choi, D.W. *Science* **236,** 589–593 (1987).
6. Mayer, M. L., Vyklicky, L. & Westbrook, G. L. *J. Physiol.* **415,** 329–350 (1989).
7. Dingledine, R. *et al. CRC Critical Rev. Neurobiol.* **4,** 1–96 (1988).
8. Chesler, M. & Chan, C. Y. *Neuroscience* **27,** 941–948 (1988).
9. Balestrino, M. & Somjen, G. G. *J. Physiol. Lond.* **396,** 247–266 (1988).
10. Kraig, R. P., Ferreira-Filho, C. R. & Nicholson, C. *J. Neurophysiol.* **49,** 831–850 (1983).
11. Krishtal, O. A., Osipchuk, Y. V., Shelest, T. N. & Smirnoff, S. V. *Brain Res.* **436,** 352–356 (1987).
12. Maycox, P. R., Deckwerth, T., Hell, J. W. & Jahn, R. *J. biol. Chem.* **263,** 15423–15428 (1988).
13. Cidon, S. & Sihra, T. S. *J. biol. Chem.* **264,** 8281–8288 (1989).
14. Grantyn, R. & Lux, H. D. *Neurosci. Lett.* **89,** 198–203 (1989).
15. Gruol, D. L., Barker, J. L., Huang, L.-Y. M. MacDonald, J. F. & Smith, T. G. *Brain Res.* **183,** 247–252 (1980).
16. Tang, C. M., Dichter, M. & Morad, M. *Soc. Neurosci. Abstr.* **15,** 326 (1989).
17. Morad, M., Dichter, M. & Tang, C. M. *Soc. Neurosci. Abstr.* **14,** 791 (1988).
18. Hamill, O. P. , Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflugers Archiv.* **391,** 85–100 (1981).
19. Pietrobon, D., Prod'hom, B. & Hess, P. *Nature* **333**, 373–376 (1988).
20. Cull-Candy, S. G., Howe, J. R. & Ogden, D. C. *J. Physiol., Lond.* **400,** 189–222 (1988).
21. Cull-Candy, S. G. & Usowicz, M. M. *Nature* **325,** 525–528 (1987).
22. Jahr, C. E. & Stevens, C. F. *Nature* **325,** 522–525 (1987).
23. Howe, J. R., Colquhoun, D. & Cull-Candy, S. G. *Proc. R. Soc.* **B233** 407–422 (1988).
24. Mutch, W. A. C. & Hansen, A. J. *J. Cereb. Blood Flow Metab.* **4,** 17–27 (1984).
25. Giffard, R. G., Monyer, H., Christine, C. W. & Choi, D. W. *Brain Res.* **506**, 339–342 (1990).
26. Kleckner, N. W. & Dingledine, R. *Science* **241,** 835–837 (1988).
27. MacDonald, J. F., Mody, I. & Salter, M. W. *J. Physiol.* **414,** 17–34 (1989).
28. Krishtal, O. A. & Pidoplichko, V. I. *Neuroscience* **5**, 2325–2327 (1980).
29. Colquhoun, D. & Sigworth, F. J. in *Single Channel Recording* (eds Sakmann, B. & Neher, E. 191–263 (New York, Plenum, 1983).
30. Patlak, J. B. *J. gen. Physiol.* **92,** 413–430 (1988).

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Migration of myoblasts across basal lamina during skeletal muscle development

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BASAL lamina is a sheet of extracellular matrix that separates cells into topologically distinct groups during morphogenesis and is thought to form a barrier to cell migration. We have examined whether, during normal muscle development, myoblasts—mononucleate muscle precursor cells[1]—can cross the basal lamina that surrounds each multinucleate muscle fibre[2]. We marked myoblasts *in vivo* by injecting replication-defective retroviral vectors encoding LacZ into muscle tissue and analysed the fate of their progeny by the expression of β-galactosidase[3,4]. A dual labelling method with broad application to retroviral lineage-marking studies was developed to ensure that most clusters of labelled cells were clones derived from a single precursor cell. Most of the myoblasts that were infected at a late stage of rat hindlimb development, when each fibre with its satellite myoblasts is individually encased in a basal lamina sheath[2,5,6], gave rise to clones that contributed to several labelled fibres. Our results show that myoblasts from healthy fibres migrate across basal lamina during normal development and could contribute to the repair of fibres damaged by injury or disease.

To mark single myoblasts and their progeny, we injected rat skeletal muscles with the recombinant BAG retroviral vector[3]. This replication-defective retrovirus is a useful marker of cell lineage, as after infection it stably integrates into the host genome, expresses β-galactosidase (β-gal) and is inherited by all progeny cells at mitosis. A histochemical analysis of serial cryostat sections of hindlimbs 2 weeks after infection revealed muscles fibres labelled with β-gal distributed in regions

TABLE 1 β-gal-labelled muscle fibres were frequently in clusters of more than one fibre

Experiment	Age (days) at		Regions with	
	Injection	Analysis	One fibre	Multiple fibres
1	9	23	9	16
2	14	38	9	10
3	14	38	5	5
4	15	37	16	22
5	16	29	1	1
6	16	29	1	3
7	19	35	0	1
		Total	41 (41%)	58 (59%)

Retroviral vector, prepared as described in the legend to Fig. 1 was injected into rat hindlimbs on or after P9, a developmental stage when the final adult number of muscle fibres has been formed and further growth of muscle is due to an increase in size of individual fibres, largely by myoblast fusion into pre-existing fibres. Three observations ensured that in our experiments new fibres were not being formed: (1) counts of muscle fibre number were similar at P9 and P30. For soleus, they were $2{,}547 \pm 233$ ($n = 3$) and $2{,}357 \pm 213$ ($n = 3$), respectively; (2) very few, if any, small-diameter nascent fibres were present after P9; (3) the greatest diameter of fibres within multifibre clones, single-fibre clones and unlabelled adjacent fibres did not differ significantly, and was 25 ± 4 μm ($n = 14$), 28 ± 4 μm ($n = 4$) and 24 ± 6 μm ($n = 94$), respectively (mean ± s.d.) at P23 (experiment 1). Serial transverse sections indicated that labelled myofibres do not bifurcate, in agreement with electron microscopic studies[13,24]. Regions of labelled fibres contained one or more fibres separated from one another by less than three fibre diameters and from all other labelled fibres by at least ten fibre diameters. In experiments 2 and 3 (also shown in Table 2), a mixture of BAG and MMuLVSVnlsLacZ was used.

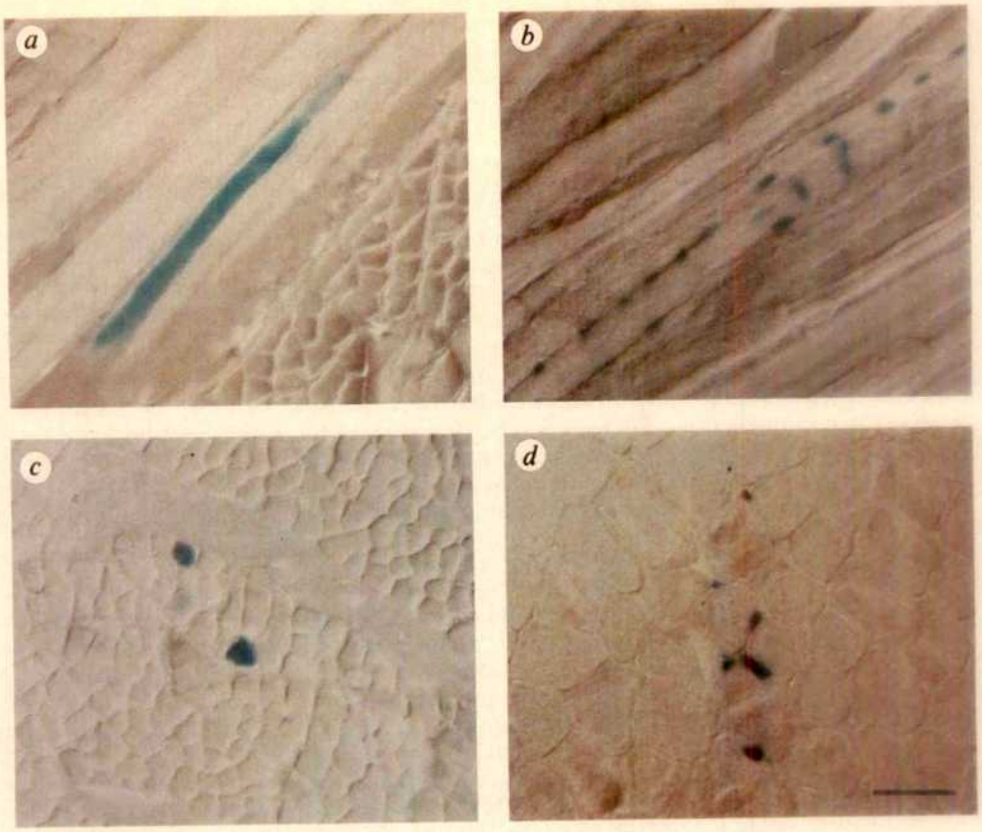

FIG. 1 Retroviral vectors target β-galactosidase to the cytoplasm or nucleus of muscle fibres *in vivo*. BAG, which expresses cytoplasmic β-galactosidase (*a*, *c*), or MMuLVSVnlsLacZ, which expresses nuclear β-galactosidase (*b*, *d*), was injected into rat hindlimb muscle at postnatal day 5 (P5) (*a*, *c*), P1 (*b*) or P15 (*d*) and analysed by serial section on P18 (*a*, longitudinal section; *c*, transverse section), P21 (*b*, longitudinal section) or P37 (*d*, transverse section), respectively. Between P21 and P37 muscle fibres increased threefold in diameter (compare *c* and *d*). Apparent nuclear diameter (*b*, *d*) varies with the plane of section. Scale bar, 50 μm. The cluster in *c* was in the lateral gastrocnemius separated by 40 fibre diameters from its closest labelled neighbour (*a*) in the biceps femoris.

METHODS. Supernatant from confluent CREBAGα or Ψ-2-21-C_3 retroviral producer cells made by transfecting BAG into the Ψ_{CRE} or pMMuLVSVnlsLacZ into the Ψ_2 ecotropic packaging cell lines respectively was collected, filtered, and concentrated by centrifugation. Wild-type helper virus was absent. Viral stock (50–200 μl) with 10 μg ml^{-1} polybrene was mixed with charcoal particles to locate the site of injection and administered as a single injection from a 26-gauge needle into the laterodorsal surface of the hindlimb of each anaesthetized Wistar rat. After 2–3 weeks, animals were analysed for the expression of β-gal. Muscle was fixed by cardiac perfusion with 4% paraformaldehyde, 0.5% glutaraldehyde, 100 mM PIPES buffer pH 7.4. After 30–60 min, lower hindlimbs were dissected free of skin and placed in the same fixative overnight at 4 °C, then in 30% sucrose PBS for 24 h at 4 °C, frozen in freezing isopentane and cut into serial 30μm sections on a Bright cryostat. In some experiments, sections were cut on unfixed tissue. All sections were postfixed in 4% paraformaldehyde in PBS washed and stained in 1 mg ml^{-1} 5-bromo-4-chloro-3-indonyl-D-galactoside (X-gal), 5 or 35 mM potassium ferri- and ferrocyanide, 1 mM $MgCl_2$ in PBS overnight at 30 °C, mounted in glycerol–PBS (9:1) and examined under bright-field optics on a Zeiss Axiophot for the presence of the blue X-gal reaction product. Charcoal particles were most frequently located between soleus and lateral gastrocnemius. The nuclear localization of nls-β-gal in tissue sections was confirmed by counterstaining with Hoechst 33258. Like cyt-β-gal, nls-β-gal diffuses within fibres and therefore labels nuclei in addition to those that encoded it[10]. Tandem arrays of 200 labelled nuclei were observed in single fibres *in vivo* (*b*). No detectable differences in frequency, morphology, size or distribution of clusters formed by BAG or MMuLVSVnlsLacZ were seen, consistent with the expectation that these vectors differ only in the distribution of β-gal to nuclear and cytoplasmic cell compartments. Clusters close to charcoal particles did not differ from those several millimetres away, suggesting that the injection did not perturb development of nearby tissue.

throughout the limb (Fig. 1), indicating that the retroviral vector could gain access to and infect muscle cells *in vivo*. Endogenous β-gal activity was negligible in muscle, as the muscles of uninjected or mock-injected animals had no labelled muscle fibres. Regions containing both labelled muscle and nonmuscle cells were not seen (data not shown), consistent with the distinct somitic origin of limb muscle in birds[7,8]. As retroviruses only infect dividing cells[9], the muscle fibres that expressed β-gal presumably resulted from infection of mitotic myoblasts and the subsequent fusion of their clonal progeny into fibres.

Labelled muscle fibres occurred either alone or in small clusters. Figure 1*a* shows a typical longitudinal section of a single labelled muscle fibre that contained β-gal detectable over a length of about 100 nuclei, equivalent to 2 mm. This finding indicated that β-gal is free to diffuse along muscle fibres[10,11], in contrast to some endogenous muscle gene products[12] and, as shown in the cluster of three fibres in Fig. 1*c*, can be analysed in transverse sections. Elsewhere in this leg, 20 regions of one

TABLE 2 Cytoplasmic and nuclear β-gal-labelled fibres segregate into distinct clusters

	Cluster number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
Experiment 1																				
Nls-β-gal fibres (*n*)	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	4	0	0	7
Cyt-β-gal fibres (*n*)	1	1	1	1	1	1	1	1	0	2	2	0	3	3	3	4	0	5	6	36
Experiment 2																				
Nls-β-gal fibres (*n*)	0	0	0	0	0	0	0	2	0	0	0									2
Cyt-β-gal fibres (*n*)	1	1	1	1	1	1	2	0	3	3	14									26

Injection of a mixture of BAG and MMuLVSVnlsLacZ retroviral vectors into rat hindlimbs revealed that fibres within clusters are generally labelled by the same vector (χ^2 analysis: $P > 0.01$, although this value is limited by the small cluster sizes). Limbs were injected at P14 with a 6:1 mixture (BAG : MMuLVSVnlsLacZ) as described in Fig. 1 and analysed in serial sections ~3 weeks later (P38).

or more labelled fibres were distributed in five muscles and separated from their nearest neighbouring region of labelled fibres by 0.47 ± 0.25 mm. Therefore clusters of labelled fibres were generally well-separated and distributed among many different muscles of the limb.

To determine whether myoblasts migrate across basal lamina, retroviral vector was injected on or after postnatal day 9 (P9). At this stage, unlike the early postnatal period (P0–P5) when myoblasts encased in the basal lamina of one fibre give rise to new fibres within that lamina[2,6], each myoblast is encased in a basal lamina in close association with a single fibre, the number of fibres typical of adult muscle has been reached[5,6], and the growth of muscle tissue occurs by the fusion of myoblasts into pre-existing fibres. An analysis of serial sections of hindlimb muscles injected on or after P9 in seven independent experiments revealed that 59% of β-gal-positive regions contained more than one labelled fibre (Table 1). Because basal lamina separated each fibre and its myoblasts from its neighbours, clusters of labelled fibres were likely to be due to the migration of myoblasts from one fibre to another.

The interpretation that myoblasts migrate relies on several labelled fibres within a cluster arising from infection of a single myoblast. Alternatively, each labelled fibre within a cluster could have resulted from a separate infection event. To rule out the latter possibility we performed two types of experiments. First, the retroviral vector was titrated so that clusters were separated by greater distances. Multifibre clusters still occurred at a high frequency (Table 1, experiments 5–7). Second, we injected a mixture of two different retroviral vectors: BAG, which expresses β-gal in the cytoplasm (cyt-β-gal) and MMuLVSVnlsLacZ (ref. 4), which targets β-gal to the nucleus because of an added N-terminal SV40 T-antigen nuclear localization sequence (nls-β-gal). These two vectors possess the same *env*-encoded protein and therefore have similar infectivity[4], yet are readily distinguishable *in vivo* (Fig. 1, compare *a*, *c* with *b*, *d*). Table 2 shows that fibres within a cluster contained either nls-β-gal or cyt-β-gal but not both. Therefore similarly labelled fibres were generally associated with one another in clusters. Given the observed cluster sizes, the probability of this association occurring if each labelled fibre arose from a separate infection is $<1/10^7$. We conclude that a cluster of similarly labelled fibres arose by infection of a single myoblast, migration of its progeny across basal lamina, and fusion into adjacent fibres.

Although the data in Table 2 strongly indicate that each cluster represents a clone, the number of clusters that could be analysed was relatively low, as most muscle growth had occurred before infection. To determine the proportion of clusters that represent single myogenic clones, we performed the dual vector experiment in rats injected immediately after birth (P0) (Table 3). At this early stage of development the rate of myoblast division is high and new fibres are still being formed, leading to a relatively high frequency of infection and formation of many large multifibre clones. Therefore, the chance of obtaining overlapping clones is greater at this stage, providing a rigorous test of whether clusters are clonal. Serial sections of two hindlimbs revealed that of 87 multifibre clusters analysed, only two clusters (<3%) contained adjacent fibres labelled with nls-β-gal and cyt-β-gal (Table 3). After correction for the probability of a double infection by the same type of vector, these data show that 91 ± 8% ($P > 0.95$) of clusters were clonal in animals infected at a titre that resulted in ~40 clusters of labelled fibres per limb. This dual retroviral vector technique should prove generally useful in lineage studies for determining the frequency with which clusters of labelled cells represent clones.

Myoblast migration across basal lamina is not a rare event. The chance that clusters were clones in animals at late stages of development when fibre formation was complete (Tables 1 and 2) is likely to be even greater than 91%, because the frequency of clusters per limb was lower (<20 per limb). As 59% of labelled regions contained multifibre clusters at this stage, we conclude that at least 50% of infected myoblasts gave rise to progeny that migrated across basal lamina and fused into neighbouring myofibres. Moreover, myoblast migration could encompass at least five separate fibres (Table 2). Whether myoblasts can migrate further remains to be determined.

TABLE 3 Most clusters of β-gal-labelled fibres are clones

	Number of multifibre clusters with		
Experiment	Nls-β-gal	Cyt-β-gal	Cyt-β-gal and nls-β-gal
1	34	11	2
2	30	10	0
Total	64 (74%)	21 (24%)	2 (2%)

Injection of a mixture of retroviral vectors at P0 and analysis at P23 when clusters of large size are obtained with high frequency, revealed that most clusters of β-gal-labelled fibres are clones. The proportion of clusters that contained both nls-β-gal (MMuLVSVnlsLacZ) and cyt-β-gal (BAG) was approximately 2%. On the basis of this value, the predicted frequencies o two infections by the same type of vector were calculated as binomia estimates and were 6% for nls-β-gal and 1% for cyt-β-gal. By adding these estimates to the observed cyt-β-gal and nls-β-gal double infection value (2%) the frequency with which regions of labelled fibres derive from single infection events and are clonal approximates 91 ± 8% ($P > 0.05$). This value corrects for the ratio of the two vectors. The error was calculated by combining the binomial variance of each estimate.

Our studies indicate that, during normal development, myoblasts frequently migrate from one muscle fibre to another at a time when the tissue is divided into a series of compartments by basal lamina. This finding is consistent with electron micrographs of undamaged muscle showing cells extending through basal lamina[13,14] and suggests that myogenic cells are present in the muscle interstitium. Previous studies suggested that myoblasts migrate[15-18], but it was unclear whether the myoblasts could traverse intact basal lamina, because in each study the tissue was traumatized. This could have led to invasion of leukocytes, which in addition to tumour cells are one of the few cell types known to cross basal lamina[19-21], that provided a migration route for myoblasts. It remains to be determined whether diffusible cues[22] or the composition of the basal lamina[23] regulate the migration of myoblasts *in vivo*, perhaps recruiting them to sites of injury.

That myoblasts can migrate from one healthy fibre to another lends promise to proposed therapies for Duchenne muscular dystrophy (DMD) based on myoblast implantation[18]. In degenerating muscle, it is possible that myoblasts migrate greater distances than those observed here. If human myoblasts, like rat myoblasts, can migrate across basal lamina, then injection of myoblasts into a few sites could constitute an efficient therapy for large regions of diseased muscle. □

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1. Mauro, A. *J. biophys. biochem. Cytol.* **9,** 493–494 (1961).
2. Kelly, A. M. & Zacks, S. I. *J. Cell Biol.* **42,** 135–153 (1969).
3. Price, J., Turner, D. & Cepko, C. *Proc. natn. Acad. Sci. U.S.A.* **84,** 156–160 (1987).
4. Bonnerot, C., Rocancourt, D., Briand, P., Grimber, G. & Nicolas, J. F. *Proc. natn. Acad. Sci. U.S.A.* **84,** 6795–6799 (1987).
5. Enesco, M. & Puddy, D. *Am. J. Anat.* **114,** 235–244 (1964).
6. Ontell, M. & Kozeka, K. *Am. J. Anat.* **171,** 133–148 (1984).
7. Chevallier, A., Kieny, M. & Mauger, A. *J. Embryol. exp. Morphol.* **41,** 245–258 (1977).
8. Christ, B., Jacob, H. J. & Jacob, M. *Anat. Embryol. Berl.* **150,** 171–186 (1977).
9. Varmus, H. E., Padgett, T., Heasley, S., Simon, G. & Bishop, J. M. *Cell* **11,** 307–319 (1977).
10. Ralston, E. & Hall, Z. W. *Science* **244,** 1066–1069 (1989).
11. Hughes, S. M. & Blau, H. M. in *The Dynamic State of Muscle Fibres,* (ed. Pette, D.) (Walter de Gruyter & Co., Berlin–New York, in the press).
12. Pavlath, G. K., Rich, K., Webster, S. G. & Blau, H. M. *Nature* **337,** 570–573 (1989).
13. Ontell, M. *Anat. Rec.* **189,** 669–690 (1977).
14. Kelly, A. M. *Anat. Rec.* **190,** 891–903 (1978).
15. Schultz, E., Jaryszak, D. L., Gibson, M. C. & Albright, D. J. *J. Muscle Res. Cell Motil.* **7,** 361–367 (1986).
16. Watt, D. J., Morgan, J. E., Clifford, M. A. & Partridge, T. A. *Anat. Embryol. Berl.* **175,** 527–536 (1987).
17. Lipton, B. H. & Schultz, E. *Science* **205,** 1292–1294 (1979).
18. Partridge, T. A., Morgan, J. E., Coulton, G. R., Hoffman, E. P. & Kunkel, L. M. *Nature* **337,** 176–179 (1989).
19. Anderson, A. O. & Anderson, N. D. *Immunology* **31,** 731–748 (1976).

20. Warfel, K. A. & Hull, M. T. *Anat. Rec.* **208,** 349–355 (1984).
21. Nakajima, M., Irimura, T., Di, F. D., Di, F. N. & Nicolson, G. L. *Science* **220,** 611–613 (1983).
22. Venkatasubramanian, K. & Solursh, M. *Devl Biol.* **104,** 428–433 (1984).
23. Ocalan, M., Goodman, S. L., Kuhl, U., Hauschka, S. D. & von der Mark, K. *Devl Biol.* **125,** 158–167 (1988).
24. Ontell, M. & Dunn, R. F. *Am. J. Anat.* **152,** 539–555 (1978).

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Two adjacent MyoD1-binding sites regulate expression of the acetylcholine receptor α-subunit gene

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SEVERAL genes encoding putative myogenic regulatory factors have been isolated on the basis of their ability to convert non-muscle cells into myoblasts[1–6]. Four of these genes code for nuclear proteins that belong to a larger family characterized by a conserved helix–loop–helix motif required for DNA-binding and dimerization[7]. At least one protein, MyoD1, can function as a transcription factor and activate muscle-specific genes during differentiation[8]. But the promoter of the δ-subunit gene of the mouse acetylcholine receptor (AChR) was recently reported to be functional in the absence of MyoD1 binding sites and it has been suggested that the genes coding for the AChR could be regulated independently of MyoD1 protein[9]. Here, we identify two functional MyoD1-binding sites in the muscle-specific enhancer of the chicken AChR α-subunit gene that are essential for full activity in transfected myotubes.

A minimal muscle-specific enhancer sequence of 36 base pairs (bp) has been defined in the chicken AChR α-subunit gene by transient expression experiments in various mouse myogenic cell lines[10–12] and in primary cultures of chicken myotubes[13]. As two potential MyoD1-binding sites are present in this sequence, we used an affinity-purified glutathione S-transferase-MyoD1 fusion protein (Glu-MyoD)[8] to probe enhancer fragments in gel retardation experiments. No shift was observed with the bacterial enzyme alone or when a fragment containing an Sp1-factor binding site was probed with Glu-MyoD (results not shown). In contrast, two retarded bands were observed for the fragment ARIIpab containing the complete enhancer (Fig. 1*a*); the lower band is labelled I and the upper II (Fig. 2*a*, lane 1). Band I alone was detected with a fragment containing only the previously characterized element ARIIb of the enhancer[13] (Fig. 2*a*, lane 4). Band II therefore probably represents a complex of two Glu-MyoD molecules, one bound to element ARIIb and a second to a more distal site.

The two binding sites were characterized more precisely by means of methylation interference experiments. Guanines, the methylation of which interfered with the formation of complexes I and II (bands I and II, Fig. 2*a*), were analysed in Fig. 2*d*, and the results represented in Fig. 1*a*. We conclude that complex I corresponds mainly (if not exclusively) to Glu-MyoD protein bound to element ARIIb. Complex II corresponds effectively to the binding of two molecules, one bound to element ARIIb and a second to a more distal site overlapping a *Pvu*II restriction site. We have called this latter site ARIIp and the downstream site ARIIb′, because it is part of element ARIIb (ref. 13).

The two binding sites are compared with the previously characterized MCK-L, MCK-R and AChR-δ sites in Fig. 1*b*[8,9]. Similarities exist in the nucleotide sequences and in the methylation interference patterns among the five sites. The close proximity of the two sites within the α-subunit gene enhancer is also remarkable. Similar motifs in both sites occur on the same side of the DNA helix, suggesting that direct interactions between two bound molecules may take place.

To evaluate the role of the two sites in α-subunit gene promoter activity, we constructed three mutants. The mutation of site ARIIp is called MUT-P, of site ARIIb′ MUT-B and combination of both mutations MUT-BP (Fig. 1*a*). The effect of MUT-B and MUT-P on Glu-MyoD binding was tested in competition experiments. In both cases, a concentration of mutated fragment at least tenfold higher than wild-type fragment was necessary to displace the same amount of protein from the ARIIb′ or ARIIp sites (Fig. 2*b* and 2*c*). We conclude that the point mutations decrease the affinity of Glu-MyoD for the α-subunit gene enhancer.

These mutations were then used to test the function of the previously characterized MyoD1 binding sites in transient expression experiments. 3T6 mouse fibroblasts were cotransfected with an expression vector for either MyoD1 or the related myogenin protein (plasmids. pEMSV-MyoD1 and pEMSV-myogenin, respectively)[1,3] and a test vector containing either

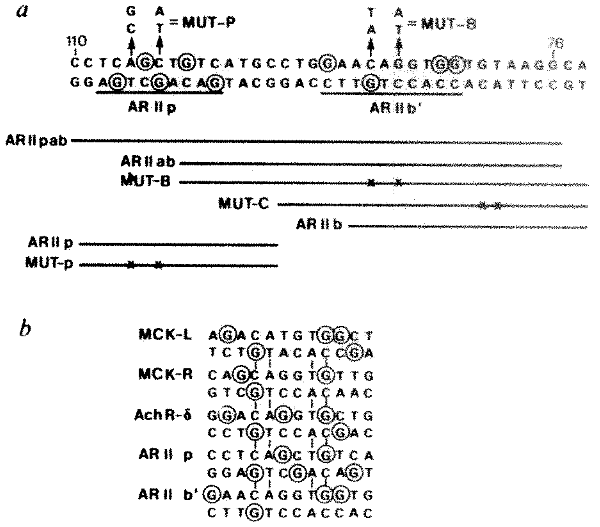

a
G A = MUT-P
C T
T A = MUT-B
A T
110
76
C C T C A G C T G T C A T G C C T G G A A C A G G T G G T G T A A G G C A
G G A G T C G A C A G T A C G G A C C T T G T C C A C C A C A T T C C G T
AR II p
AR II b′
AR II pab
AR II ab
MUT-B
MUT-C
AR II b
AR II p
MUT-p
b
MCK-L A G A C A T G T G G C T
T C T G T A C A C C G A
MCK-R C A G C A G G T G T T G
G T C G T C C A C A A C
AchR-δ G G A C A G G T G C T G
C C T G T C C A C G A C
AR II p C C T C A G C T G T C A
G G A G T C G A C A G T
AR II b′ G A A C A G G T G G T G
C T T G T C C A C C A C

FIG. 1 DNA sequence of the AChR α-subunit gene enhancer. *a*, The sequence represents nucleotides −110 to −74 relative to the transcription start site of the chicken AChR α-subunit gene[10]. Guanines whose methylation interfere with Glu-MyoD binding are circled, mutated base pairs in MUT-B and MUT-P are indicated with arrows. MUT-BP is the combination of both mutations. Double-stranded oligonucleotide fragments used in gel retardation and competition experiments are indicated below the sequence. Crosses represent mutated base pairs. A4-bp linker sequence was added 5′ to facilitate subsequent cloning or 3′-end labelling of the oligonucleotide fragments. *b*, Comparison of MyoD1 binding sites in muscle-specific genes. Symbols are as in (*a*). Sequences for MCK-L and MCK-R are from ref. 8 and that for AChR-δ is from ref. 9. The conserved CA–TG motif is indicated.

wild-type or mutated promoter sequences of the AChR α-subunit gene (nucleotides −842 to +20 in pαAChCAT+; ref. 10) linked to the chloramphenicol acetyltransferase (CAT) gene (Fig. 3*a*). A minimal promoter containing only 45 bp upstream of the transcription start point was not transactivated under these conditions (not shown). In contrast, the pαAChCAT+ construct was transactivated by both MyoD1 and myogenin. Mutation of either site ARIIp or ARIIb′ led to a significant inhibition of transactivation. Specifically, mutation of ARIIb′ (MUT-B) had a stronger inhibitory effect than that of ARIIp (MUT-P). Mutation of both sites (MUT-BP) led to an almost complete loss of transactivation. These results show that at least two myogenic factors, MyoD1 and myogenin can transactivate the α-subunit gene promoter through the ARIIp and ARIIb′ sites.

MyoD1 expression activated the α-subunit gene promoter as well as constructs containing several copies of the ARIIb element in front of the herpes simplex virus thymidine kinase (TK) promoter in several cell types: non-myogenic 3T6 mouse fibroblasts (Fig. 3*a*, and data not shown) or CV1 monkey epithelial cells (not shown) and myogenic C2.7 myoblasts (Fig. 3*b*). Figure 3*b* illustrates the dependence of the level of transactivation on the copy number of the MyoD1-target (compare ARIIb-3TKM containing three copies of ARIIb′ and ARIIb-10TKM with 10 copies). Noteworthy is the fact that the presence of detectable levels of MyoD1 protein and messenger RNA in this cell line does not correlate with transcriptional activity[14,15]. We speculate that expression of high levels of MyoD1 from the introduced vectors could either titrate out repressor molecules that interact with MyoD1, or might exhaust a repressing pathway, functioning for instance through covalent modifications of MyoD1.

We next investigated whether the two MyoD1 binding sites are critical for α-subunit gene promoter activity in the more physiological context of primary cultures of chick myotubes (Fig. 3*c*). Mutation of each MyoD1 site separately had only a moderately negative effect on promoter activity as determined by CAT-assay (~twofold). But the combination of both mutations produced a strong decrease in promoter activity (~20-fold). This effect was observed as early as one day after plating (results not shown), most probably perturbing the binding of factors appearing very early during *in vitro* differentiation of myoblasts. Thus, mutations of only four base pairs in sites ARIIb′ and ARIIp strongly affect AChR α-subunit gene promoter function, highlighting the importance of the factors binding to these two sites in the tissue-specific expression of the promoter.

Data from (1) *in vitro* binding experiments using purified Glu-MyoD fusion protein, (2) transactivation experiments with expression vectors for MyoD1 and myogenin in several cell lines, and (3) the effect of specific point mutations of the MyoD1 binding sites on promoter activity, implicate myogenic factors of the MyoD1 family in the regulation of the AChR α-subunit promoter. Gel retardation and competition experiments with the ARIIp and ARIIb′ sites suggest the presence in chicken myotubes of several MyoD1-like factors with closely related specificities (J.P., unpublished results). An attractive possibility is that among the different myogenic factors, some regulate 'constitutive' muscle genes such as muscle creatine kinase[8] and others regulate 'electric activity-dependent' genes such as the AChR genes. This latter type of factor could form a

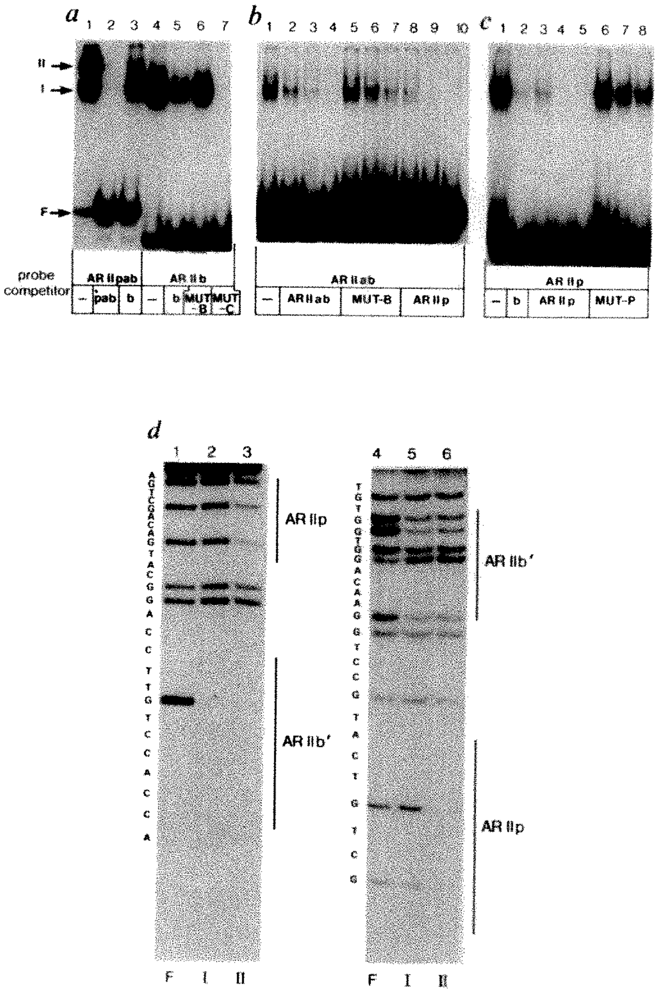
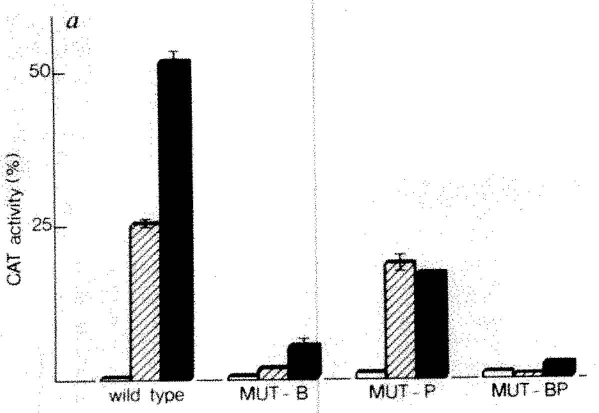


FIG. 2 MyoD1 binding to the AChR α-subunit gene enhancer. *a*, Probe ARIIpab (see Fig. 1) was used in lanes 1–3, probe ARIIb in lanes 4–7. A 200-fold molar excess of unlabelled fragment ARIIpab is used in lane 2, fragment ARIIb in lanes 3 and 5, fragment MUT-B in lanes 6 and fragment MUT-C in lane 7. One microgram affinity-purified Glu-MyoD protein was used in each experiment. *b*, Probe ARIIab (Fig. 1) was used in all lanes. A 20-fold, 100-fold and 200-fold molar excess was used for each competitor fragment: fragment ARIIab in lanes 2–4, fragment MUT-B in lanes 5–7 and fragment ARIIp in lanes 8–10; 0.4 μg affinity purified Glu-MyoD protein was used in each experiment in the presence of 1 μg poly(dI.dC). *c*, Probe ARIIp (Fig. 1) was used in all lanes. A 20-fold, 100-fold and 200-fold molar excess was used for each competitor fragment: fragment ARIIp in lanes 3–5 and fragment MUT-P in lanes 6–8. A 200-fold molar excess of fragment ARIIb was used in lane 2. 0.4 μg of Glu-MyoD protein was used in each experiment. *d*, Methylation interference experiments. Fragment ARIIpab 5′ labelled at the lower strand (Fig. 1*a*) was loaded in lanes 1–3, and at the upper strand in lanes 4–6. Free DNA (F, Fig. 2*a*) is loaded in lanes 1 and 4, DNA from complex I in lanes 2 and 5, and DNA from complex II in lanes 3 and 6.

METHODS. *a–c*, Incubation and gel electrophoresis conditions were as described[6]. Oligonucleotide fragments were labelled by fill-in labelling with Klenow polymerase. *d*, Experiments were performed as described[13], and the conditions for incubation and gel electrophoresis were as described for *a–c*. Fragment ARIIpab was used as probe. The methylated fragments were used in gel retardation experiments with 4 μg Glu-MyoD in the presence of 1 μg poly(dI.dC). The DNA was eluted from the retardation gels and subsequently cleaved at methylated purines with NaOH. DNA cleaved at methylated guanines was loaded onto a 15% denaturing polyacrylamide gel.



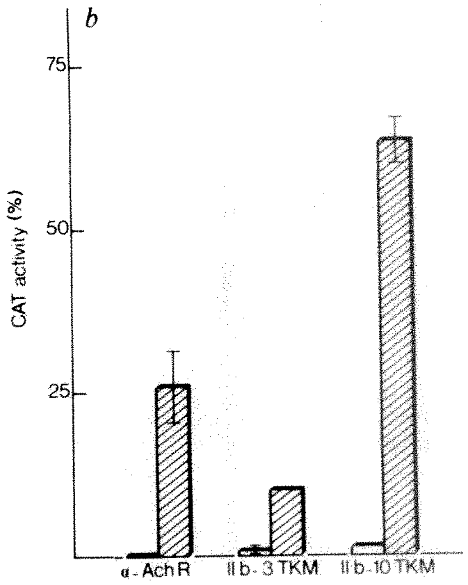
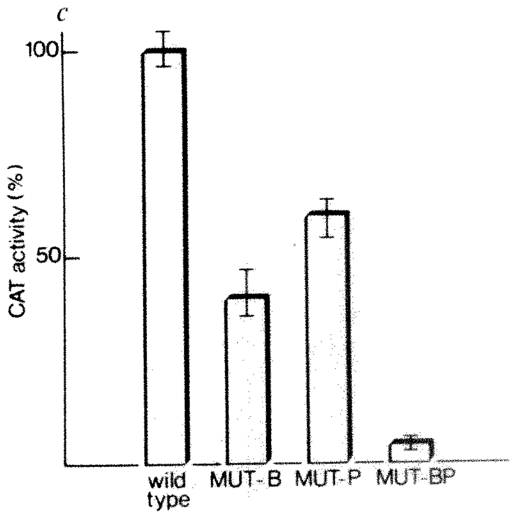


FIG. 3 Transient expression experiments. *a*, Transactivation of the AChR α-subunit gene promoter by MyoD1 and myogenin. 376 fibroblasts were used as host cells. Open bars, test plasmid only; hatched bars, test plasmid with MyoD1; filled bars, test plasmid with myogenin. The data are expressed as per cent of pSVECAT activity determined in parallel. Each experiment was performed in duplicate. Bars indicate variation between duplicate dishes. *b*, Transactivation of the AChR α-subunit gene promoter and the ARIIbTKM constructs. C2.7 cells were used as host cells. Open and hatched bars, as *a*. Experiments were run in duplicate as in (*a*) and the results are expressed as a percentage of PSVECAT activity, which was determined in parallel. ARIIb-3TKM contains three copies of ARIIb and ARIIb-10TKM contains 10 copies of ARIIb cloned in the SalI site of TKM[20]. The plasmid pαACh842/CAT contains nucleotides −842 to +3 of the promoter linked to the CAT gene[13]. *c*, Transient expression experiments in primary cultures of chicken myotubes. The activity of the mutants is expressed as per cent of wild-type activity (that is, pαAChCAT+). The values are the average of triplicate experiments. METHODS *a*, *b*, Mouse 3T6 fibroblasts (*a*) were grown in DMEM supplemented with 7% fetal calf serum (Gibco) and cotransfected with 2 μg of test plasmid and 2 μg of expression vector using the Ca-phosphate coprecipitation method[18]. Total amount of transfected DNA was kept at 6 μg with pEMS-Vscribe vector[1]. Cells were collected 36 h after transfection and CAT activity was determined using the enzymatic assay described in ref. 19. The pαACh-CAT+ construct was used as test vector: it contains bp −842 to +20 of the AChR α-subunit promoter linked to the bacterial CAT gene[10]. Point mutations (Fig. 1*a*) were introduced in this background by site directed mutagenesis (Amersham). The sequence was verified by dideoxysequencing (Pharmacia) over at least 200 bp preceding the transcription start point. pEMSV-MyoD1 and pEMSV-myogenin were used as expression vectors[1,3]. They contain the MSV LTR linked to the cDNA of MyoD1 and myogenin respectively. C2.7 myoblasts (*b*) were kept in DMEM supplemented with 20% FCS and collected before confluence. *c*, Myoblasts of 11-day-old chicken embryos were transfected 3 h after plating with 2 μg plasmid by calcium phosphate co-precipitation and cells were collected 3 d later[13].



transcriptionally active complex with a labile protein whose synthesis is repressed by electrical activity or be itself subject to such regulation[16]. Characterization of the myogenic factors that interact with the AChR genes should help to determine their role in differential expression of these genes in junctional and extra-junctional nuclei during endplate formation[17]. ☐

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1. Davis, R. L., Weintraub, H. & Lassar, A. B. *Cell* **51,** 987-1000 (1987).
2. Pinney, D. F., Pearson-White, S. M., Konieczny, S. F., Latham, K. E. & Emerson, C. P. Jr *Cell* **53,** 781–793 (1988).
3. Wright, W. E., Sassoon, D. A. & Lin, V. K. *Cell* **56,** 607–617 (1989).
4. Braun, T., Buschhausen-Denker, G., Bober, E., Tannich, E. & Arnold, H. H. *EMBO J.* **8,** 701–709 (1989).
5. Rhodes, S. J. & Konieczny, S. F. *Genes Dev.* **3,** 2050–2061 (1989).
6. Colmenares, C. & Stavnezer, E. *Cell* **59,** 293–303 (1989).
7. Murre, C., McCaw, P. S. & Baltimore, D. *Cell* **56,** 777–783 (1989).
8. Lassar, A. B. *et al. Cell* **58,** 823–831 (1989).
9. Baldwin, T. J. & Burden, S. J. *Nature* **341,** 716–720 (1989); correction *Nature* **345,** 364 (1990).
10. Klarsfeld, A., Daubas, P., Bourachot, B. & Changeux, J.-P. *Molec. cell. Biol.* **7,** 951–955 (1987).
11. Daubas, P. *et al. Nucleic Acids Res.* **16,** 1251–1271 (1988).
12. Wang, Y., Xu, H. P., Wang, X. M., Ballivet, M. & Schmidt, J. *Neuron* **1,** 527–534 (1988).
13. Piette, J., Klarsfeld, A. & Changeux, J.-P. *EMBO J.* **8,** 687–694 (1989).
14. Tapscott, S. J. *et al. Science* **242,** 405–441 (1988).
15. Montarras, D., Pinset, C. Chelly, J., Kahn, A. & Gros, F. *EMBO J.* **8,** 2203–2207 (1989).
16. Duclert, A., Piette, J. & Changeux, J.-P. *Proc. natn. Acad. Sci. U.S.A.* **87,** 1392–1395 (1990).
17. Changeux, J. P. *et al. NATO ASI Series* Vol. H32 (Springer, Berlin, 1989).
18. Graham, F. L. & Vander Eb, A. J. *Virology* **52,** 456–467 (1973).
19. Seed, B. & Sheen, J.-Y. *Gene* **67,** 271–277 (1988).
20. Tronche, F. *et al. Molec. Biol. Med.* (in the press).

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Genetic organization of a chimpanzee lentivirus related to HIV-1

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SIMIAN immunodeficiency viruses have been isolated from four species of monkey, the 'captive' macaque[1] and mangabey[2-4] and the 'feral' African green monkey[5] and mandrill[6]. While none of these viruses is a replica of HIV-1, the macaque[7] and mangabey[8] viruses represent correct genetic models for HIV-2, possessing exactly the same complement of genes. Recently a lentivirus has been identified in two wild chimpanzees (*Pan troglodytes troglodytes*) in Gabon, west equatorial Africa, and isolated from one of them[9]. This virus is referred to as $\mathrm{SIV_{CPZ}}$. Sera from these animals cross reacted with all the HIV-1 proteins including the envelope glycoproteins. Here, we describe the molecular cloning and sequencing of an infectious proviral clone of $\mathrm{SIV_{CPZ}}$. The overall genetic organization was the same as that of HIV-1, but phylogenetic analysis revealed that the sequence was more divergent than any HIV-1 sequence reported so far. The *vpu* gene product, found only in the type 1 viruses, was particularly different (64% divergent to $\mathrm{HIV\text{-}1_{BRU}}$) suggesting that the $\mathrm{SIV_{CPZ}}$ represents a distinct subtype. These findings indicate that there is a larger pool of simian lentiviruses than previously suspected and revives debate as to the origins of HIV-1.

A phage λ genomic library was constructed from total DNA of $\mathrm{SIV_{CPZ}}$-infected human lymphocytes. On screening with a HIV-1 probe, three recombinants (1a, 30 and 46a) carrying a complete $\mathrm{SIV_{CPZ}}$ provirus were isolated. The three clones had identical *Sma*I, *Xba*I, and *Bam*HI restriction profiles as well as eight *Hin*dIII restriction sites, one of which was lacking in clone 46a. Fragments containing the complete provirus from clones 1a and 46a were subcloned into plasmid pKP55 using unique *Eco*RI restriction sites in the cellular flanking sequences. On transfection into the human colon carcinoma cell line SW480, both clones were able to produce virus, with reverse transcriptase titres peaking at 7 and 14 days respectively for clone 1a and 46a (data not shown). Clone 1a virus proved to be infectious either by co-cultivation of the transfected SW480 cells with human peripheral blood mononuclear cells (PBMCs) or by passage of cell-free supernatant to donor PBMCs. Because of this and its more rapid kinetics, clone 1a was selected for sequencing (see Fig. 1 legend, ref. 10).

The $\mathrm{SIV_{CPZ}}$ genome proved to be more closely related to HIV-1 group than to any of the simian lentiviruses. Thus the organization of the $\mathrm{SIV_{CPZ}}$ genome was 5′ *gag-pol-vif-vpr-tat-rev-vpu-env-nef* 3′. The presence of a HIV-1-specific *vpu* gene and the absence of a *vpx* gene, common to HIV-2 and most SIVs, clearly show that $\mathrm{SIV_{CPZ}}$ is the first non-human lentivirus to have the same genetic structure as HIV-1. Table 1 gives the percentage amino acid sequence similarities for pairwise comparisons of all gene products of $\mathrm{SIV_{CPZ}}$ to those from other human and simian lentiviruses. The values confirm that quantitatively $\mathrm{SIV_{CPZ}}$ has, as nearest relative, the HIV-1 virus group. Nonetheless, the $\mathrm{SIV_{CPZ}}$ genome is more divergent than any of the HIV-1 isolates, including African and European strains, and yet is also equidistant to all of these.

To better appreciate the degree of divergence of the $\mathrm{SIV_{CPZ}}$ genome, a pairwise comparison of the $\mathrm{SIV_{CPZ}/HIV\text{-}1_{BRU}}$[11]

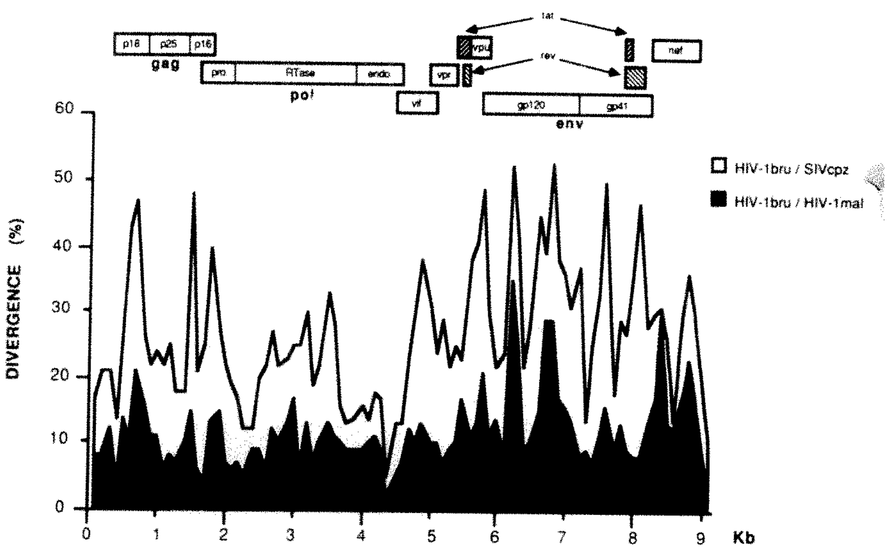

FIG. 1 Organization of the aligned $\mathrm{SIV_{CPZ}}$ genome and sequence divergence with respect to $\mathrm{HIV\text{-}1_{BRU}}$. The $\mathrm{HIV\text{-}1_{BRU}}$, $\mathrm{HIV\text{-}1_{MAL}}$ and $\mathrm{SIV_{CPZ}}$ nucleotide sequences were aligned. All gaps and corresponding sequences were eliminated. The percentage sequence variation was calculated for the $\mathrm{HIV\text{-}1_{BRU}/HIV\text{-}1_{MAL}}$ (dark shaded area in graph) and $\mathrm{HIV\text{-}1_{BRU}/SIV_{CPZ}}$ (lightly shaded area in graph) pairs over a window of 100 bases. The windows did not overlap. Gaps apart the BRU/MAL and BRU/CPZ genomes are 11.8% and 26.9% divergent respectively. In every case the $\mathrm{SIV_{CPZ}}$ sequence was always the more divergent of the two. Some parts of the *gag* gene, notably p18 and p16 are almost as divergent as parts of *env* at the nucleic acid level.

METHODS. Total DNA was partially digested with *Bgl*II. Fragments of 10–20 kilobases (kb) were selected from a sucrose density gradient, purified and ligated into *Bam*HI-cleaved λEMBL3 DNA. After packaging *in vitro*, a phage library of 1.5×10^6 clones was plated out on *Escherichia coli* P2 392. Plaques were screened *in situ* with a complete probe of $\mathrm{HIV\text{-}1_{OYI}}$[22]. Three clones carrying full length $\mathrm{SIV_{CPZ}}$ proviruses were studied in detail. Clones 1a and 46 were subcloned into the pKP55 vector (supplied by Keith Peden). Plasmid DNA (10 μg) was transfected onto 2×10^6 SW480 cells by a modified $\mathrm{CaPO_4}$ method[25]. Near the peak of reverse transcriptase, supernatant was taken, clarified and used to infect PBMCs. A third of the SW480 cells were irridiated and co-cultivated with PBMCs. Reverse transcriptase in the culture supernatants confirmed that clone 1a represented an infectious molecular clone. Clone 1a DNA was sonicated as described[11] and fragments of 600–1000 base pairs (bp) isolated and cloned into the dephosphorylated *Sma*I site of M13mp18 RF DNA. Templates were sequenced using Taq polymerase and fluorescent primers and the products resolved using an Applied Biosystems 370A DNA sequencer[10]. The sequence was compiled as described[26]. Two regions were poorly represented in the contig. Accordingly, oligonucleotide primers were synthesized allowing amplification of regions 2084–2273 and 6215–7132. These regions were amplified by the polymerase chain reaction[27] (PCR) from 100 ng of plasmid 1a DNA in 10 cycles. Amplified DNA was cleaved by *Bam*HI (this restriction site having been added to the PCR primers) and cloned into the *Bam*HI site of M13mp18 RF DNA and sequenced. For each region two or more clones were sequenced, ruling out Taq polymerase error. Each base in the $\mathrm{SIV_{CPZ}}$ sequence was sequenced on average 8.9 times. The flanking cellular DNA represented non-coding sequences. Like HIV-1, a 5-bp direct repeat of cellular DNA was formed on integration. The $\mathrm{SIV_{CPZ}}$ provirus was 9,811 bp long with the following base composition: T 22.7%; C 18.7%; G 24.5%; A 34.2%. This sequence has been filed with the EMBL database under accession number X52154.

TABLE 1 Amino acid homology between HIV/SIV isolates (%)

								ENV			
SIV_{CPZ} compared with:	*gag*	*pol*	*vif*	*vpr*	*tat*	*rev*	*vpu*	*env*	SU	TM	*nef*
$HIV\text{-}1_{BRU}$	74.8	84.0	67.2	79.2	63.9	54.3	35.8	62.7	59.5	66.9	70.2
$HIV\text{-}1_{OYI}$	74.7	83.7	67.7	80.2	65.6	55.2	38.5	63.5	61.5	65.9	74.1
$HIV\text{-}1_{MAL}$	73.4	84.1	67.7	79.2	65.5	55.2	37.3	65.8	63.4	69.0	72.9
$HIV\text{-}1_{ELI}$	74.3	83.5	67.2	83.3	61.6	56.4	37.5	65.1	65.0	65.2	69.7
$HIV\text{-}2_{ROD}$	56.2	57.2	35.1	47.3	36.1	32.0	—	37.0	34.2	40.6	36.5
SIV_{MAC}	54.9	57.4	35.8	49.0	39.8	32.0	—	35.3	31.3	40.5	36.1
SIV_{AGM}	55.9	60.4	33.7	—	34.1	39.8	—	38.6	33.2	45.9	45.0
SIV_{MND}	52.1	58.1	30.6	34.4	32.2	28.0	—	32.7	28.1	38.3	41.8

Similarity between pairs of protein sequences using SIV_{CPZ} as reference. Sequences were aligned using the program of Wilbur and Lipman[21] (k-tuple size 1, window 20, gap penalty 2). The sequences (and geographical origin) are $HIV\text{-}1_{BRU}$ (France)[11], $HIV\text{-}1_{OYI}$ (Gabon)[22], $HIV\text{-}1_{MAL}$ and $HIV\text{-}1_{ELI}$ (Zaire)[12], $HIV\text{-}2_{ROD}$ (Cape Verde)[13], SIV_{MAC} 142 (Boston)[7], SIV_{AGM} TYO (Kenya)[23] and SIV_{MND} GB-1 (Gabon)[24].

nucleotide sequences is given in Fig. 1. The $HIV\text{-}1_{MAL}$[12]/$HIV\text{-}1_{BRU}$ pair is also given for comparison. Until now, $HIV\text{-}1_{MAL}$ was the most divergent type 1 genome to be completely sequenced. Several regions of the chimpanzee sequence were more than 50% divergent with respect to $HIV\text{-}1_{BRU}$. Some parts of the *gag* gene were almost as varied as the hypervariable regions in *env*. It is also apparent that the SIV_{CPZ} genome was not simply a more divergent HIV-1 isolate (Fig. 1). There were a number of regions, notably around the protease, *vpu* and gp41, which were much more variable than would have been predicted by an extrapolation of the $HIV\text{-}1_{BRU}$/$HIV\text{-}1_{MAL}$ pattern. This would suggest that a given gene was not evolving in a regular manner. Averaged over the entire genome, the SIV_{CPZ} sequence encoded roughly 2.3 times as many transitions and transversions as the $HIV\text{-}1_{MAL}$ sequence, using $HIV\text{-}1_{BRU}$ as reference.

At the 5′ end of the SIV_{CPZ} genome the long terminal repeat encoded all the important transcriptional and regulatory elements, but the R-U5 boundary was not clear. The upper stem, bulge and loop of the TAR region were all perfectly conserved. In the base of the stem there were a number of compensating mutations which preserved the secondary structure. The primer binding site (complementary to $rRNA_3^{Lys}$) and polypurine tract were identical to those of HIV-1. Of the *gag* proteins, p18 and p16 were highly divergent (39% and 37% respectively at the amino acid level) as opposited to p25 (10%). The *tat* sequence is interesting because the first coding exon encoded an additional threonine residue following the nucleolar targeting sequence, resulting in a 73-residue protein.

Without doubt the most surprising finding was an extremely divergent but intact *vpu* protein sequence (64% divergent). Alignment of the *vpu* sequences of four HIV-1 strains as well as the SIV_{CPZ} sequence emphasized a conserved dodecapeptide (residues 47–58, Fig. 2*a*). In fact only sequence homology in this region was significant. Alignment of the remaining parts of the *vpu* sequence resulted in scores considered as background. Many of the differences must be conservative, however, because hydropathicity profiles were quite similar (Fig. 2*b*). Indeed the hydrophobic amino-terminal sequence is clearly discernible in SIV_{CPZ} (residues 3–26). These data indicate that the SIV_{CPZ} genome does in fact encode a *vpu* gene.

Two further observations emphasized the uniqueness of the SIV_{CPZ} *vpu* gene. First, part of the *vpu* gene was amplified by the polymerase chain reaction (PCR) from the PBMCs of four Gabonese HIV-1 seropositives. On sequencing all four *vpu* protein sequences proved to be more closely related to those of $HIV\text{-}1_{BRU}$ and $HIV\text{-}1_{MAL}$ than to SIV_{CPZ} (data not shown). Second, the *tat* and *rev* protein sequences of HIV-1 and HIV-2 are 57% and 55% divergent[13]. Yet comparative analyses of the two *tat*[14] and two *rev*[15] proteins indicated that they were not functionally equivalent. It is therefore possible that the SIV_{CPZ} *vpu* sequence cannot complement correctly a HIV-1 vpu^- genome. Because the HIV-1 *vpu* protein has a positive role in the budding and maturation of the virion[16], the SIV_{CPZ} equivalent could result in altered virus maturation or release.

Given the highly conserved dodecapeptide probably representing the active site, the protein sequence databases were searched for any possible similarities to the SIV_{CPZ} *vpu* sequence. Intriguingly a reasonable score was associated with the Epstein-Barr virus BNLF-1 coding sequence (residues 165–238). The region of similarity covered both the hydrophobic N-terminus and the *vpu* dodecapeptide. The hydropathic profile of this region of the BNLF-1 protein sequence closely resembles that of the *vpu*. The BNLF-1 gene encodes a late membrane protein which, at high concentration, is toxic for cell lines in culture[17].

Radioimmunoprecipitation analysis of labelled SIV_{CPZ} indicated that the surface glycoprotein (SU) and glycoprotein precursor were somewhat larger than for HIV-1 reference strains[9]. The SIV_{CPZ} SU protein sequence is as long and encodes as many potential N-linked glycosylation sites, as all other HIV-1 SU sequences. Therefore the discrepancies between the observed relative molecular masses may be due to the degree of glycosylation. All the cysteine residues N-terminal to the membrane-spanning region were conserved. The V3 loop, albeit very divergent, still encoded the Gly-Pro-Gly tripeptide, indicating that it must serve some important biological or structural function.

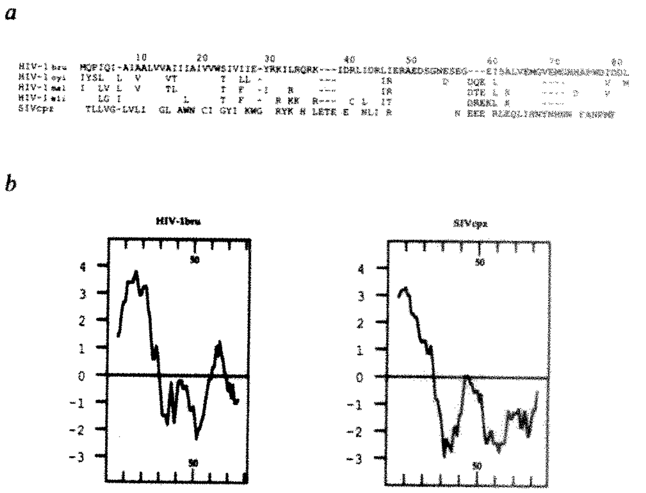

FIG. 2 Characterization of SIV_{CPZ} *vpu* sequence. *a*, Sequence alignment and *b*, hydropathic profiles of the SIV_{CPZ} *vpu* protein sequence with those of other HIV-1 sequences. The alignments were made as described in Table 1 and are given using the single-letter amino acid code. The hydropathic profiles were calculated using the program of Kyte and Doolittle[25]. The y-axis represents the hydrophobicity (+) or hydrophilicity (−) of peptide segments in arbitrary units. The x-axis represents sequence length.

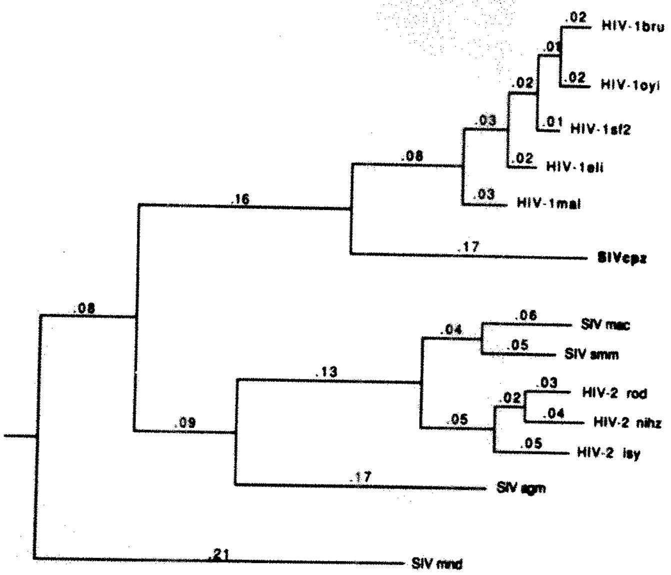

FIG. 3 Minimum length evolutionary tree for the gag region showing the relationship of SIV_{CPZ} to the known HIV and SIV lentiviruses. The total number of sites examined was 1,473 of which 967 were variable. The consistency index was 0.675. The lengths of the horizontal lines are proportional to the minimum number of single nucleotide substitutions required to generate the observed variation (also indicated by the numeric branch lengths above each line). The additional sequences not referred to in Table 1 are $\text{HIV-1}_{\text{SF2}}$[29], SIV_{SMM} (sooty mangabey)[8], $\text{HIV-2}_{\text{NIHZ}}$[29] and $\text{HIV-2}_{\text{ISY}}$[29].
METHODS. The protein sequences were aligned using a clustering and pattern analysis algorithm from the amino-acid class covering utility (AACC)[19]. Corresponding nucleotide sequence alignments were derived from the protein alignments and subjected to multiple parsimony using the PAUP program[20]. The bootstrap analysis used the DNABOOT program in the PHYLIP 3.21 package[30].

The transmembrane protein (TM) is nearly as divergent as the SU (Table 1). This is unusual for HIV-1 but not for HIV-2. The SIV_{CPZ} *rev* protein is also quite divergent (Fig. 1 and Table 1). As most of the *rev* coding region overlaps with the C-terminal half of TM, this could explain why the TM sequence is so variable. Finally, in every HIV-1 sequence to date, the *env* stop codon (TAA) forms part of the optimal *nef* start sequence (TAAGATGG in which the 'Kozak' motif is underlined). For SIV_{CPZ} an additional 12 bases separate the two open reading frames.

As anticipated, the envelope variable regions were indeed hypervariable. Apart from the cysteine and a few flanking residues, as well as the conserved V3 GPG tripeptide, it seems that virtually any hydrophilic amino acid sequences can be accommodated. If so, then why do isolates from AIDS patients still show sequence similarities in these regions? A simple explanation could be that the global AIDS epidemic is derived from one particular strain, reflected by the $\text{HIV-1}_{\text{BRU-MAL}}$ family, the differences among them representing only 15–20 years of divergence. In a similar manner, many of the North American strains are closely related to strain HIV-1_{MN}[18], perhaps because this, or a related strain, was one of the first to enter the United States.

A phylogenetic tree analysis[19,20] was undertaken of the SIV_{CPZ} nucleotide sequence. The minimal tree for the *gag* region is given in Fig. 3. The topology of the tree was the same for all regions studied. Moreover, bootstrap analysis of the *nef* tree (not shown) confirmed this position for the SIV_{CPZ} sequence in 50 out of 50 trials. It is clear that SIV_{CPZ} branched before any of the HIV-1 strains sequenced so far. The chimpanzee and HIV-1 sequences were more divergent than SIV_{MAC} or SIV_{SMM} were to HIV-2 (Fig. 3). In view of the particularity of SIV_{CPZ}, its original designation[9], it might be more informative to refer to this virus as chimpanzee immunodeficiency virus, CIV.

In Gabon, only 2 out of 83 chimpanzees tested were seropositive, indicating that SIV_{CPZ} is not widely dispersed in this region. Given their troop behaviour and fear of water, it is possible that SIV_{CPZ} infection may be highly restricted geographically. Of more than 250 chimpanzees caught over the last 15–20 years in West Africa, none was seropositive. This might help explain the absence of naturally infected chimpanzees in captivity in the United States as virtually all are of West African origin (A. Prince, personal communication).

That the two chimpanzees were both infants and in relatively good health should not be surprising, for if they suffered from anything resembling AIDS or AIDS-related complex, they would not have long survived predators in the jungle. Intriguingly none of the 'feral' SIVs, the African green monkey and mandrill viruses, is associated with disease, perhaps for the same reason. Ironically simian AIDS has only been described among captive monkeys where living conditions and general hygiene are much better. Consequently, discussion of the relative pathogenicity of SIV_{CPZ} is not prudent. It is, however, an intriguing question as there is relatively little AIDS in this region of Africa.

Inevitably the similarity between the HIV-1 and SIV_{CPZ} genomes elicits speculation as to their evolutionary relationship and the origins of the current AIDS epidemic. Obviously there are HIV-1-like viruses in chimpanzees. It is not possible to conclude that SIV_{CPZ} was the precursor to HIV-1, if indeed infection ever passed in that direction. Even given this premise, the *vpu* data indicate that SIV_{CPZ} was not the immediate precursor. Clearly more data on SIV_{CPZ} prevalence and genetics would be useful.

In only a few years, lentiviruses have been isolated from African green monkeys, mandrills and now chimpanzees. It may be only a matter of time before other HIV-1-like lentiviruses will be isolated. An extensive serological study of simians, perhaps using the HIV-1 *vpu* sequence might yield just such a virus. □

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1. Daniel, M. D. *et al. Science* **228,** 1201–1204 (1985).
2. Lowenstine, L. J. *et al. Int. J. Cancer* **38,** 563–574 (1986).
3. Murphey-Corb, M. *et al. Nature* **321,** 435–437 (1986).
4. Fultz, P. N. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 5286–5290 (1986).
5. Ohta, Y. *et al. Int. J. Cancer* **41,** 115–122 (1988).
6. Tsujimoto, H. *et al. J. Virol.* **62,** 4044–4050 (1989).
7. Chakrabarti, L. *et al. Nature* **328,** 543–547 (1987).
8. Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. *Nature* **339,** 389–392 (1989).
9. Peeters, M. *et al. AIDS* **3,** 625–630 (1989).
10. Connell, C. *et al. Bio Techniques* **5,** 342–348 (1987).
11. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S & Mizon, M. *Cell* **40,** 9–17 (1985).
12. Alizon, M., Wain-Hobson, S., Montagnier, L. & Sonigo, P. *Cell* **46,** 63–74 (1986).
13. Guyader, M. *et al. Nature* **326,** 662–669 (1987).
14. Emerman, M., Guyader, M., Montagnier, L., Baltimore, D & Muesing, M. *EMBO J.* **6,** 3755–3760 (1987).
15. Malim, M. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 8222–8226 (1989).
16. Klimkait, T., Strebel, K., Hoggan, M. D., Martin, M. A. & Orenstein, J. M. *J. Virol.* **64,** 621–629 (1990).
17. Hammerschmidt, W., Sugden, B. & Baichwal, V. R. *J. Virol.* **63,** 2469–2475 (1989).
18. Gurgo, C. *et al. Virology* **164,** 531–536 (1988).
19. Smith, R. F. & Smith, T. F. *Proc. natn. Acad. Sci. U.S.A.* **87,** 118–122 (1990).
20. Smith, T. F., Srinivasan, A., Schochetman, G., Marcus, M. & Myers, G. *Nature* **333,** 573–575 (1988).
21. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 726–730 (1983).
22. Huet, T., Dazza, M. C., Brun-Vézinet, F., Roelants, G. E. & Wain-Hobson, S. *AIDS* **3,** 707–715 (1989).

23. Fukasawa, M. *et al. Nature* **333,** 457–461 (1988).
24. Tsujimoto, H. *et al. Nature* **341,** 539–541 (1989).
25. Chen, C. & Okayama, H. *Molec. cell. Biol.* **7,** 2745–2752 (1987).
26. Staden, R. *Nucleic Acids Res.* **10,** 4731–4751 (1982).
27. Saiki, R. K. *et al. Science* **239,** 487–491 (1988).
28. Kyte, J. & Doolittle, R. *J. molec. Biol.* **157**, 105–132 (1982).
29. Myers, G. *et al. Human Retroviruses and AIDS* (Los Alamos National Laboratory, New Mexico, 1989).
30. Felsenstein, J. *A. Rev. Genet.* **22,** 521–565 (1988).

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How different eukaryotic transcriptional activators can cooperate promiscuously

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A STRIKING characteristic of many different eukaryotic transcriptional activators is their ability to activate gene expression synergistically. Thus, for example, the rat glucocorticoid receptor and the yeast activator GAL4 cooperatively activate transcription of a mammalian gene bearing binding sites for each of the proteins[1]: activation by both activators is greater than the sum of the effects of each working alone. It would seem unlikely that these two proteins from such different organisms directly interact; rather, the idea has been suggested that these and at least some other eukaryotic activators can work synergistically by simultaneously touching some part of the transcriptional machinery[2]. An important prediction of this idea is that synergy between two such activators would be seen under conditions where each is present at concentrations sufficiently high to saturate its site on DNA. In this paper we use transcription *in vitro* to confirm that prediction using a derivative of the yeast activator GAL4 and the mammalian transcription factor ATF. The accompanying paper[3] describes a similar conclusion comparing the effects of singly and multiply bound GAL4 molecules.

We have previously demonstrated that a GAL4 derivative synthesized in and purified from *Escherichia coli* can function synergistically with the mammalian activator ATF in a HeLa cell nuclear extract[4]. Here we study this phenomenon further and show that synergism is observed under conditions in which the binding sites for ATF and the GAL4 derivative are saturated.

The template used in these transcription experiments contained a single ATF site immediately adjacent to the TATA box of the adenovirus E4 gene; five GAL4 sites, termed 17-mers, were positioned 160 base pairs (bp) upstream (see Fig. 1). Transcription was carried out in HeLa cell nuclear extracts depleted of endogenous ATF by sequence-specific DNA affinity chromatography[5,6]. The *E. coli*-derived GAL4 derivative GAL4(1–147)+AH (ref. 7) and purified mammalian ATF were added to the extracts as indicated and transcription was measured by primer extension[4]. Binding of GAL4(1–147)+AH and ATF in the extracts was examined by DNase I footprinting using a ^{32}P-labelled template DNA fragment.

Increasing amounts of purified ATF or GAL4 (1–147)+AH were added to an ATF-depleted extract and transcriptional activity was determined in the presence of an excess of the other activator. The autoradiograph of such a transcription experiment is shown in Fig. 1 and the data quantitated and plotted in Fig. 2. Figure 2*a* shows the transcription elicited by various amounts of GAL4(1–147)+AH in the presence and absence of excess ATF. This figure also shows the occupancy of the GAL4 sites as measured by DNase I footprinting. Figure 2*b* shows the transcription elicited by ATF in the presence and absence of excess GAL4(1–147)+AH. This figure also shows the occupancy of the ATF site as measured by DNase I footprinting.

Two results are evident from the experiment of Fig. 2. First, in each case, activation paralleled site filling as assayed by DNase I footprinting. Second, taking Fig. 2*a* and *b* together, addition of an excess of one activator resulted in a synergistic increase in transcription at all concentrations of the other activator. In particular, as shown by the bar graph of Fig. 3, this synergy was observed when both activators were present at concentrations sufficient to saturate their sites on DNA. In Fig. 3 the hatched bar represents transcription at the highest concentration of ATF tested in the absence of GAL4(1–147)+AH; the grey bar represents transcription at the highest concentration of GAL4(1–147)+AH tested in the absence of ATF; the black bar represents transcription at the highest concentrations of both activators. The white bar represents the expected value if the response to the two activators were additive. Because the black bar is greater than the white bar we say that ATF and GAL4(1–147)+AH are working synergistically.

As purified ATF comprises multiple DNA-binding species[6], we cannot assess the relative contribution of each polypeptide to the observed transcriptional activity. But this does not alter our conclusions, which are based on the observation that the GAL4 and ATF binding sites are saturated.

Our experiments describe synergistic activation under conditions that render cooperative DNA binding of the activators irrelevant, that is, at a concentration of each activator sufficient to fill its sites. We have also obtained similar results by adding

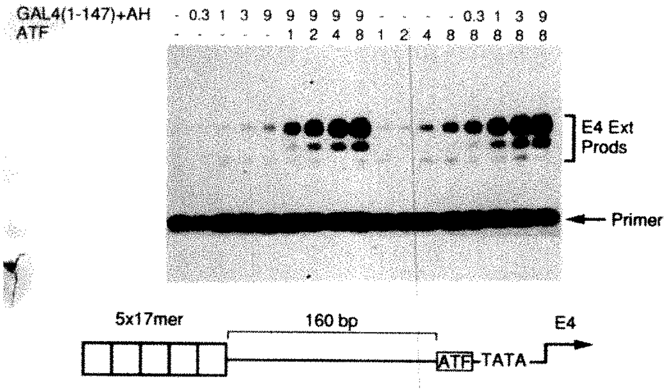
GAL4(1-147)+AH - 0.3 1 3 9 9 9 9 9 - - - - 0.3 1 3 9
ATF - - - - - 1 2 4 8 1 2 4 8 8 8 8 8
E4 Ext Prods
Primer
5x17mer
160 bp
E4
ATF
TATA

FIG. 1 Titration of ATF and GAL4 in an ATF-depleted nuclear extract. Transcription mixtures contained ATF-depleted nuclear extracts and the indicated concentrations (DNase I footprinting units) of either ATF or GAL4(1–147)+AH. Transcription products were measured by primer extension. The primer-extension products were fractionated on a denaturing polyacrylamide gel and made visible by autoradiography. The DNA template used in the experiment is shown schematically below the autoradiograph.

METHODS. The preparation of ATF oligonucleotide column and purification of ATF were as previously described[5,6]. Nuclear extract (2 ml) was adjusted to 0.3 M KCl, passed through 1 ml of the ATF resin pre-equilibrated with buffer D (ref. 15) containing 0.3 M KCl. The flow-through was collected and reloaded onto the ATF oligonucleotide resin. The second flow-through was collected as the ATF-depleted nuclear-extract and dialysed against buffer D containing 0.05 M KCl. The ATF-depleted nuclear extracts contain no detectable ATF activity as measured by DNase I footprinting and transcription assays; these extracts could support normal levels of transcription from the adenovirus major late promoter, indicating that they contained all general transcription factors (data not shown). All other methods were as previously described[4].

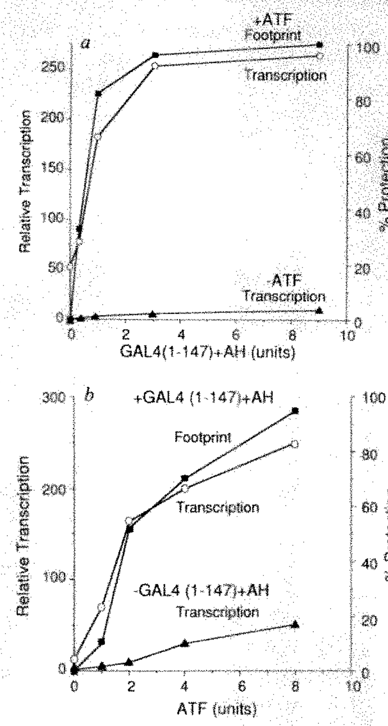

FIG. 2 Graphical comparison of DNase I footprinting (■) and transcription (○, ▲). *a*, GAL4 titration plus or minus saturating ATF. The autoradiograph shown in Fig. 1 was scanned with a densitometer and the normalized areas for each point plotted. The Y axis indicates relative transcription and per cent protection assayed in parallel by DNase I footprinting; a value of one has been arbitrarily assigned to the level of transcription seen in the absence of either activator. The *x* axis indicates the amount of GAL4(1–147) + AH in DNase I footprinting units. *b*, ATF titration plus or minus saturating GAL4(1–147) + AH. The figure is organized as in *a* except that titration was with ATF.

METHODS. Autoradiographs were scanned with a Helena densitometer and quantitated. For the transcription data (Fig. 1) dilutions of the primer were used to create a standard curve. For the DNase I footprints (not shown) the ratio of the protected bands to the unaffected bands was determined at each point in the titration. This ratio was divided by that determined in a control reaction lacking proteins. Plotted is (1 − the quotient) × 100, the per cent of DNA protected by the protein.

GAL4(1–147) + AH to a crude HeLa cell nuclear extract in which endogenous ATF is present at a saturating concentration (ref. 4, and data not shown). We imagine that this synergy is a consequence of interaction of each of the bound activators with some component or components of the transcriptional machinery rather than with each other. We might further consider two alternatives: the activators might simultaneously contact the same target as illustrated in Fig. 4, or they might contact different targets and affect different steps in the activation process. The accompanying paper describes synergistic activation by multiply-bound copies of a single activator, a result suggesting multiple contacts with a single target or with multiple copies of a single target. Previous reports[8,9] suggest that both of the activators used in these experiments interact with the transcription factor, TFIID.

We note that, should the activators simultaneously contact a third component, they could bind DNA cooperatively, a phenomenon that could be relevant at low activator concentration. Cooperative DNA binding has a crucial role in the action of λ repressor[10], for example, but in that case the cooperativity results from direct repressor–repressor interaction[11].

Many eukaryotic promoters contain multiple binding sites for various activators, the combinations of which vary amongst different promoters. In several cases it has been shown that mutation of one or more of these binding sites can be compensated for by duplicating one or more of the remaining sites

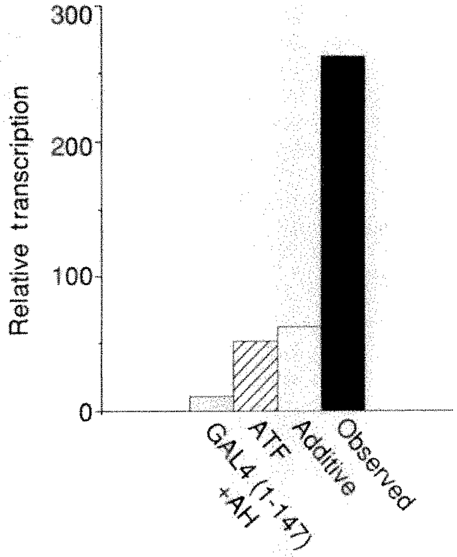

FIG. 3 Bar graph of synergism. The autoradiograph shown in Fig. 1 was scanned with a densitometer and the relative areas plotted as a bar graph. The *y* axis is relative transcription; the amount of transcription stimulated by the highest concentrations of ATF and GAL4(1–147) + AH are indicated; additive indicates the sum of the transcription signals elicited by GAL4(1–147) + AH and ATF assayed separately; observed indicates the amount of transcription actually measured when the two activators were assayed in the same reaction mixture.

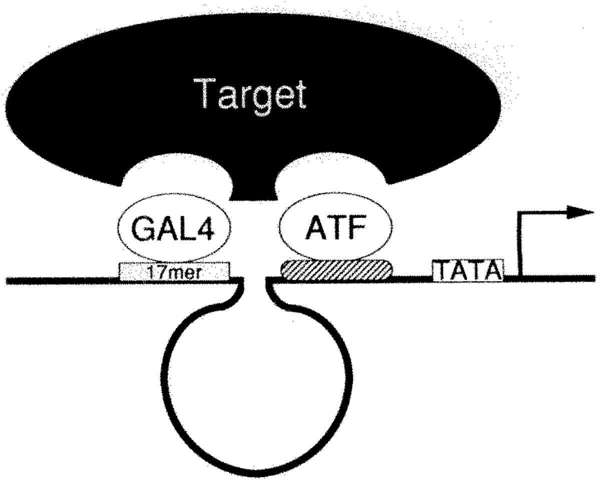

FIG. 4 Schematic representation of heterologous activators simultaneously contacting a common target. The diagram shows molecules of GAL4-(1–147) + AH and ATF, bound to their sites on DNA, simultaneously contacting an as-yet-unidentified target protein; the intervening DNA loops out to accommodate the reaction. Our experiments indicate that as many as five activators may simultaneously interact with the target.

(see, for example, refs 12-14). We imagine that at least some of the activators that bind to these sites in eukaryotic promoters work synergistically by the mechanism described in this paper. ☐

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1. Kakidani, H. & Ptashne, M. *Cell* **52,** 161-167 (1988).
2. Ptashne, M. *Nature* **335,** 683-689 (1989).
3. Carey, M. F., Lin, Y.-S., Green, M. R. & Ptashne, M. *Nature* **345,** 361-364 (1990).
4. Lin, Y.-S., Carey, M., Ptashne, M. & Green, M. R. *Cell* **54,** 659-664 (1988).
5. Lin, Y.-S. & Green, M. R. *Proc. natn. Acad. Sci. U.S.A.* **86,** 109-113 (1989).
6. Hai, T. Y., Liu, F., Allegretto, E. A., Karin, M. & Green, M. R. *Genes Dev.* **2,** 1216-1226 (1988).
7. Giniger, E. & Ptashne, M. *Nature* **330,** 670-672 (1987).
8. Horikoshi, M., Carey, M., Kakidani, H. & Roeder, R. G. *Cell* **54,** 665-669 (1988).
9. Horikoshi, M., Hai, T., Lin, Y.-S., Green, M. R. & Roeder, R. G. *Cell* **54,** 1033-1042 (1988).
10. Johnson, A. D., Pabo, C. O. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5061-5065 (1979).
11. Hochschild, A. & Ptashne, M. *Cell* **44,** 681-687 (1986).
12. Ondek, B., Gloss, L. & Herr, W. *Nature* **333,** 40-45 (1988).
13. Fromenthal, C., Kanno, M., Nomiyama, H. & Chambon, P. *Cell* **54,** 943-953 (1988).
14. Schaffner, G., Schirm, S., Muller-Baden, B., Weber, F. & Schaffner, W. *J. molec. Biol.* **201,** 81-90 (1988).
15. Dignam, J. D., Martin, P. L., Shastry, B. S. & Roeder, R. G. *Meth. Enzym.* **101,** 582-598 (1983).

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A mechanism for synergistic activation of a mammalian gene by GAL4 derivatives

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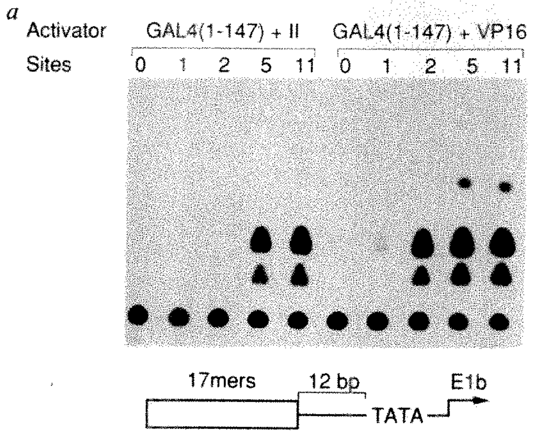
IN prokaryotes and eukaryotes many gene activators work synergistically. For example, two dimers of λ repressor interact to promote binding of these proteins to DNA, a reaction that is crucial at the repressor concentrations found in lysogens[1]. In this case one of the bound dimers activates transcription, evidently by touching RNA polymerase[2]. In another example, the yeast transcriptional activator GAL4, which can stimulate transcription in many eukaryotes, binds to multiple sites on DNA to activate transcription synergistically; the presence of two such sites can elicit a level of transcription more than twice that found with a single site[3]. In this paper we show that synergistic activation by each of several GAL4 derivatives involves a mechanism different from that illustrated by the λ repressor: multiple activator molecules can work synergistically under conditions in which their binding sites on DNA are saturated. The accompanying paper shows that under similar conditions of activator excess, GAL4 derivatives work synergistically with a heterologous mammalian gene activator[4]. These results support the idea that eukaryotic activators can cooperate not by directly interacting but by simultaneously touching some component(s) of the transcriptional machinery.

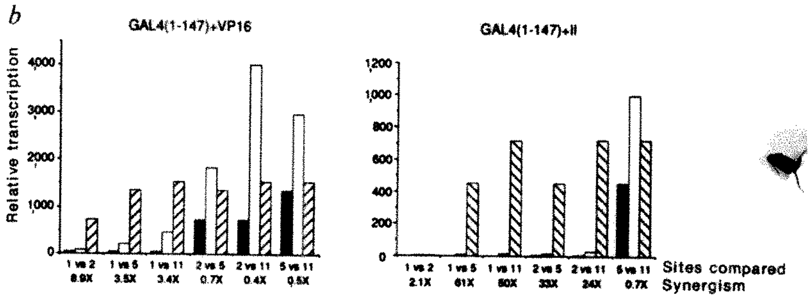
Figure 1 compares activation *in vivo* using GAL4(1-147)+II (ref. 5) and GAL(1-147)+VP16 (ref. 6), a weak and a strong activator, respectively. These GAL4 derivatives bear identical DNA-binding regions, but differ in their activation regions: II is derived from GAL4 and VP16 from the herpes viral protein of that name. Each template contains the TATA region from the adenovirus E1b promoter fused to the reporter gene for chloramphenicolacetyltransferase CAT (ref. 7) and bears either 0, 1, 2, 5 or 11 17-base pair (bp) GAL4 binding sites[8] upstream from the TATA box. These templates were co-transfected into Chinese hamster ovary (CHO) cells with an effector plasmid directing the synthesis of one or the other activator, and CAT activity was taken as a measure of transcription. Figure 1*a* shows CAT assays obtained in such an experiment, and the tabulation in Fig. 1*b* compares the response of pairs of templates bearing different numbers of GAL4 DNA binding sites. For each pair in Fig. 1*b*, the black bar represents transcription from the template bearing the smaller number of sites and the hatched bar that from the template bearing the larger number of sites; the white bar represents in each case the expected value if the response to the multiple bound activators were additive with respect to the template with the smaller number of sites. Thus the ratio of the heights of the cross-hatched to the white bars gives a measure of synergism. Using GAL4(1-147)+VP16, for example, the response to five sites is at least 3.5 times greater than five times the response of a template bearing a single site. Using GAL4(1-147)+II, the response of a template bearing five sites is at least 33-fold greater than 2.5 times the response of a template bearing two sites. We take these results to mean that both activators are working synergistically (cooperatively). Two additional points to note are: first, the differences in the responses to the strong and weak activators diminish as the number of sites increases. Thus for example, on a template bearing only two sites the difference is at least 100-fold, whereas on a template bearing eleven sites the difference is only about twofold. Second, for both the strong and the weak activators, as the number of sites is increased, a point is reached at which synergy is no longer observed; this point requires more sites for the weak activator (5-11) than it does for the strong activator (2-5).

The results of the experiment described in Fig. 1*c* argue that the experiments of Fig. 1*a* were carried out at conditions in which the activator-binding sites were saturated. Figure 1*c* shows a typical titration curve generated by co-transfecting cells with a constant amount of reporter plasmid and increasing amounts of effector plasmid. As the effector concentration increased, transcription rose and reached a plateau at between 5 and 10 μg of effector plasmid added. The experiments of Fig. 1*a* and *b* were all carried out using 10 μg of effector plasmid, an amount sufficient for maximal transcription.

The experiments of Figs 2 and 3, carried out *in vitro*, further support the idea that synergy is observed under conditions in which the activators saturate their DNA-binding sites. For these experiments we used GAL4 derivatives synthesized in and purified from *Escherichia coli*. In addition to GAL4(1-147)+VP16 we used two weak activators: GAL4(1-147)+AH (ref. 9) bears a short peptide designed to form an amphipathic α-helix and GAL4(1-147) bears a cryptic activating region that weakly activates transcription in mammalian cell extracts[10] but not in mammalian and wild-type yeast cells. Each template bears 0, 1, 2, 5 or 9 GAL4-binding sites upstream of the adenovirus E4 TATA box[10]. Figure 2 shows the use of a gel retardation assay to measure the amount of three GAL4 derivatives required to fill their sites on each of the reporter templates used in a subsequent transcription experiment. The transcription experiment was then performed in HeLa cell nuclear extracts in the presence of excess activator; the product mRNAs were measured by primer extension analysis (Fig. 3*a*). Figure 3*b* summarizes the data shown in Fig. 3*a*. For all three activators, synergism was readily apparent (see, for example, the response of one versus nine sites using GAL4(1-147)+AH). Also as observed *in vivo*, as the number of GAL4 binding sites increases, the differences in response to a weak (GAL4(1-147)+AH) and the strong (GAL4(1-147)+VP16) activator greatly diminishes; at even greater numbers of sites the response to GAL4(1-147) approaches that seen with the other activators (data not shown).

A further demonstration that the experiment in Fig. 3*a* was done under conditions in which the activator binding sites were saturated is provided by the experiments of Fig. 3*c* and *d*. Figure 3*c* describes activation by increasing amounts of GAL4(1-147)+VP16 on templates bearing various numbers of binding sites and Fig. 3*d* shows the results of parallel DNase I footprinting





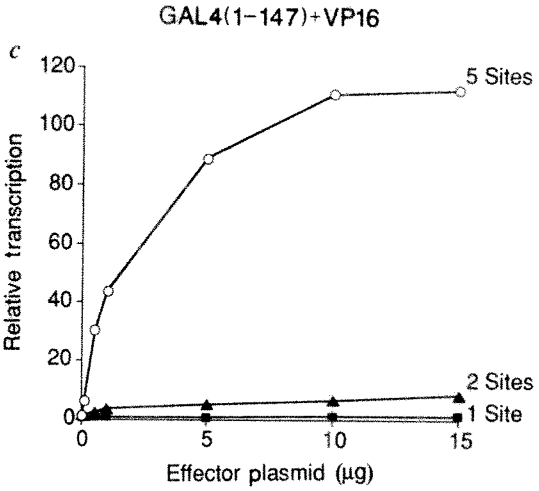


FIG. 1 Transcriptional synergism *in vivo*. *a*, Templates bearing the indicated number of 17-mers cloned 12 bp upstream of the E1b TATA box were transfected into CHO cells with effector plasmids directing the synthesis of GAL4(1-147) + II and GAL4(1-147) + VP16. After 60 h cells were collected and assayed for CAT activity. An autoradiogram of a typical experiment is shown. The template is indicated below. *b*, Bar graphs representing the data in *a*. Solid bars represent the amount of transcription found with the lower number of sites, hatched bars represent the amount found with the higher number of sites. The white bars are the expected value if the sites were having a simple additive effect. We compared each possible combination of sites, for example one versus two sites. The degree of synergism, indicated below each comparison, is defined as the ratio of the observed (cross hatched) to what is predicted if the sites were having an additive effect on transcription (white). *c*, A typical titration of effector plasmid. Increasing amounts of GAL4(1-147) + II effector plasmid were co-transfected into CHO cells with a constant amount of a reporter plasmid containing the indicated number of GAL4-binding sites. CAT activity was measured and plotted against the amount of effector DNA. The level of transcription in the absence of effector plasmid was assigned a value of 1.

METHODS. Transfections: 5 μg of reporter plasmid and the inidicated amounts of effector plasmid were transfected onto CHO cells using the DEAE-Dextran technique[13] and CAT activity was measured and quantitated as described previously[14]. Cloning: the plasmid pE1bCAT (ref. 7) contains the adenovirus E1b TATA box cloned immediately upstream of the CAT gene. The reporter templates contained 1, 2, 5 and 11 tandem GAL4-binding sites cloned into the *Hin*cII site of pE1bCAT.

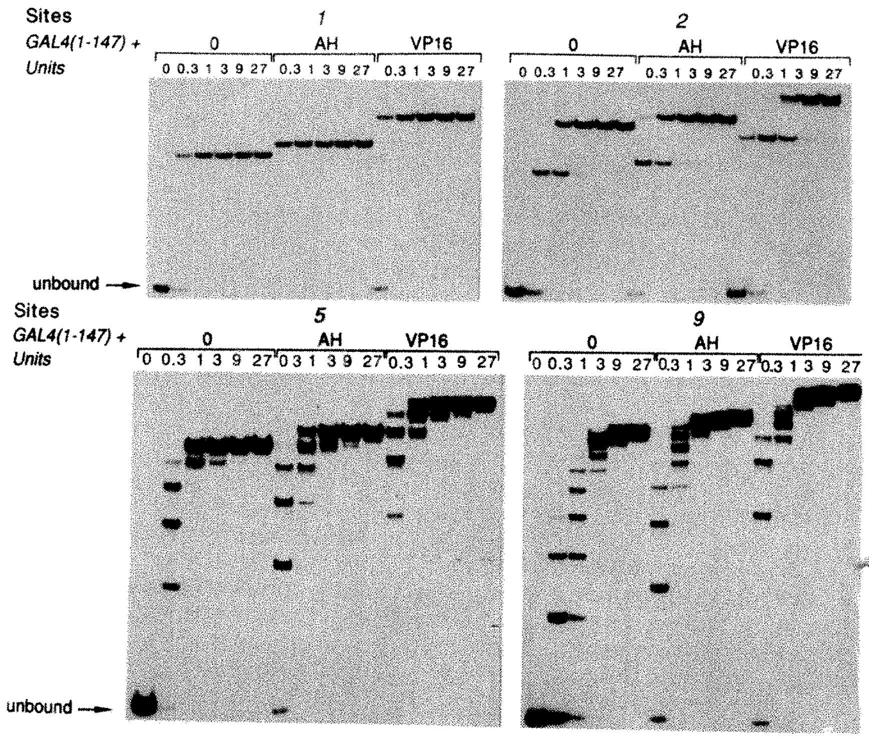
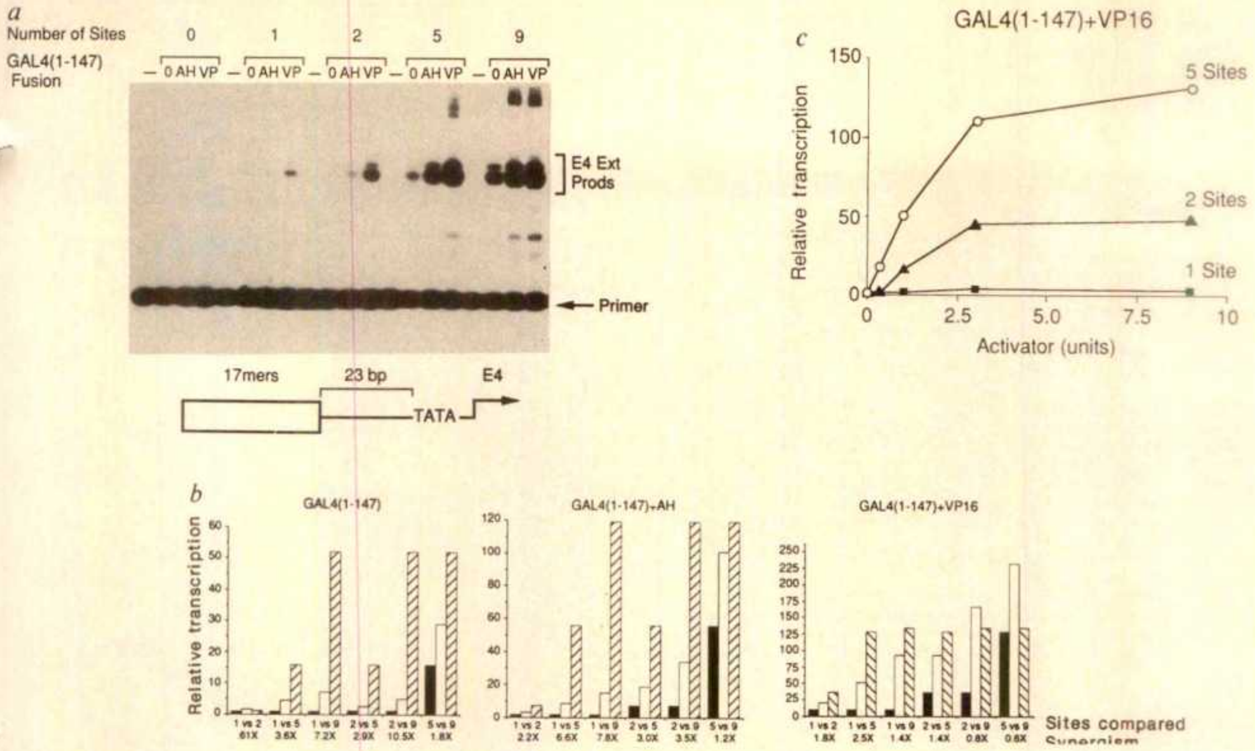


FIG. 2 Normalizing DNA binding of the activators to the reporter templates for *in vitro* transcription. The indicated GAL4 derivatives were titrated in reactions containing ^{32}P-labelled restriction fragments bearing the indicated number of GAL4-binding sites (17-mers). The conditions resembled those used in the *in vitro* transcription reactions but lacked HeLa nuclear extract. The resulting complexes were fractionated by electrophoresis though non-denaturing polacrylamide gels as described previously[8]. The experiment in Fig. 3*a* was carried out under conditions in which the template sites were saturated with activator as determined by this assay. At low protein concentrations each activator generates the number of complexes expected from the number of template sites. The larger activators give rise to complexes with slower electrophoretic mobilities than the smaller activators.



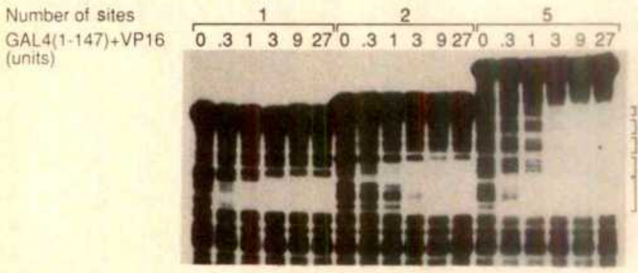


FIG. 3 Transcriptional synergism *in vitro*. *a*, Templates bearing the indicated number of 17-mers cloned 23 bp upstream of the E4 TATA box were transcribed in HeLa cell nuclear extract in the absence (–) or presence of 9 units of GAL4(1–147), designated 0, GAL4(1–147) + AH, designated AH, or GAL4(1–147) + VP16, designated VP. The reaction products were measured by primer extension[10] and fractionated on a polyacrylamide–urea gel. An autoradiograph of the gel is shown. The primer and E4 extension products are indicated to the right of the autoradiograph. The multiple products are the result of transcription initiating at different points. *b*, The autoradiograph in *a* was scanned with a Helena densitometer, using dilutions of the primer to create a standard curve, and plotted as a bar graph. Each activator is represented by a separate graph. The background transcription in the presence of no sites was assigned a value of 1 and subtracted from the stimulated signal, the bars are described in the legend to Fig. 1. *c*, GAL4(1–147) + VP16 was titrated in transcription reactions on templates bearing the indicated number of binding sites. The autoradiographs were scanned with a Helena densitometer and plotted against the level of activator. The side-by-side comparison in *a* was made with 9 units of activator, an amount on the plateau level of the curve. *d*, DNase I footprinting assays were performed in the nuclear extract in parallel to the transcription assays shown in *c*.

METHODS. Activator purification, *in vitro* transcription and DNase I footprinting assays have been described[10,15,16]. Cloning: the plasmid pΔ-38 (ref. 10) contains the E4 TATA box, start site and 250 bp of coding sequence cloned into the *Sma*I site of pGem3. The plasmids pG_1E4T, pG_2E4T, pG_5E4T and pG_9E4T were constructed by inserting one, two, five and nine tandem GAL4-binding sites into the *Hin*cII site of pΔ-38, 23 bp upstream from the E4 TATA box (E4T).

assays. The amount of protein added in the experiment of Fig. 3*a* is at least threefold above that required for maximal transcription and is sufficient to fill the template sites in a DNase I footprinting experiment.

We conclude that the GAL4-derived activators studied here can work cooperatively under conditions in which their sites on DNA are saturated. The accompanying paper[4] shows that this statement can be extended to describe the synergistic effect of GAL4 and a mammalian transcriptional activator, ATF. These results are consistent with the idea that DNA-bound GAL4 molecules simultaneously contact a component(s) of the transcriptional apparatus as shown schematically in Fig. 4. This idea would explain the evidently promiscuous properties of various eukaryotic activators (see, for example, refs 11 and 12): any set of activator molecules that could simultaneously interact with some part of the transcriptional machinery would work cooperatively. If this idea is correct, our data suggest that somewhere between 5–10 molecules of activator can contact

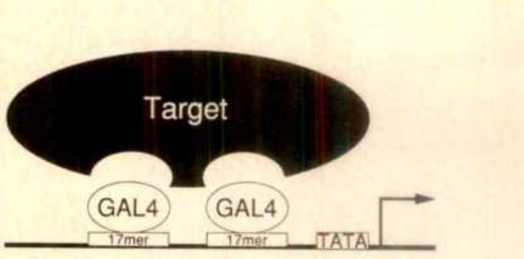


FIG. 4 Schematic representation of multiple activators simultaneously contacting a common target. Two molecules of a GAL4 derivative, bound to adjacent sites on DNA, simultaneously contact an as-yet-unidentified target protein. The interaction energies would add, leading to an exponential increase in the affinity of the target for multiple activators; the exponential increase in affinity would generate a synergistic increase in transcription. Our experiments suggest that more than two, and possibly as many as 5–10 molecules of certain activators may simultaneously interact with the target. The target is shown as a single molecule although there may be multiple targets that interact with one another.

the transcriptional target simultaneously. We note that according to this scenario the activator(s) might, like λ repressor, bind DNA cooperatively when present at sufficiently low concentration, but the effect would be mediated not by direct touching between the activators, but rather by indirect interaction. □

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1. Johnson, A. D., Pabo, C. O. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5061-5065 (1979).
2. Hochschild, A., Irwin, N. & Ptashne, M. *Cell* **32,** 319-325 (1983).
3. Giniger, E. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 383-386 (1987).
4. Lin, Y.-S., Carey, M. F., Ptashne, M. & Green, M. R. *Nature* **345,** 359-361 (1990).
5. Ma, J. & Ptashne, M. *Cell* **48,** 847-853 (1989).
6. Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. *Nature* **335,** 563-564 (1988).
7. Lillie, J. W. & Green, M. R. *Nature* **338,** 39-44 (1989).
8. Carey, M., Kakidani, H., Leatherwood, J., Mostashari, F. & Ptashne, M. *J. molec. Biol.* **209,** 423-432 (1989).
9. Giniger, E. & Ptashne, M. *Nature* **330,** 670-672 (1987).
10. Lin, Y. S., Carey, M., Ptashne, M. & Green, M. R. *Cell* **54,** 659-664 (1988).
11. Davidson, I., Xiao, J. H., Rosales, R., Staub, A. & Chambon, P. *Cell* **54,** 931-942 (1988).
12. Strahle, U., Schmid, W. & Schutz, G. *EMBO J.* **11,** 3389-3395 (1988).
13. Lopata, M. A., Cleveland, D. W. & Sollner-Webb, B. *Nucleic Acids Res.* **12,** 5705-5717 (1984).
14. Lin, Y.-S. & Green, M. R. *Nature* **340,** 656-659 (1989).
15. Chasen, D., Leatherwood, J., Carey, M., Ptashne, M. & Kornberg, R. *Molec. cell. Biol.* **9,** 4746-4749 (1989).
16. Carey, M., Leatherwood, J. & Ptashne, M. *Science* **247,** 710-712 (1990).

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CORRECTION

Muscle-specific gene expression controlled by a regulatory element lacking a MyoD1-binding site

Timothy J. Baldwin & Steven J. Burden

Nature 341, 716-720 (1989).

WE reported that muscle-specific gene expression could be conferred by a *cis*-acting regulatory region in the murine acetylcholine receptor δ-subunit gene that lacks a MyoD1-binding site. Subsequently, we found that the sequence of several plasmid constructs used in these experiments was different from that reported. We reconstructed these plasmids, verified their sequence and, as studied previously, analysed their ability to confer muscle-specific expression on the c-*fos* basal promoter (FBP), a tissue-nonspecific promoter. We demonstrated previously that nucleotides −148/−108 of the δ-subunit gene are necessary for muscle-specific expression and bind myotube nuclear proteins distinct from MyoD1. We concluded that the MyoD1-binding site (−23/−15) is not sufficient to confer muscle-specific expression, and that an activity distinct from MyoD1 could bind to a *cis*-acting region necessary for muscle-specific expression. We confirm these results. We also reported that the FBP alone conferred low-level expression in C2 myotubes and 3T3 cells, whereas −148/−95/FBP and −148/−53/FBP conferred high level expression only in C2 myotubes. Therefore we concluded that −148/−95, a *cis*-acting region that lacks a MyoD1-binding site, was sufficient to confer muscle-specific expression upon a tissue-nonspecific promoter. We now find that the FBP alone and −148/−95/FBP confer identical low-level expression in C2 myotubes and 3T3 cells. In addition, −148/−53/FBP confers high-level expression not only in C2 myotube cultures, but also in 3T3 cells. Consequently, we have no evidence that the *cis*-acting regulatory region that lacks a MyoD1-binding site (−148/−95) is sufficient to confer muscle-specific expression. □

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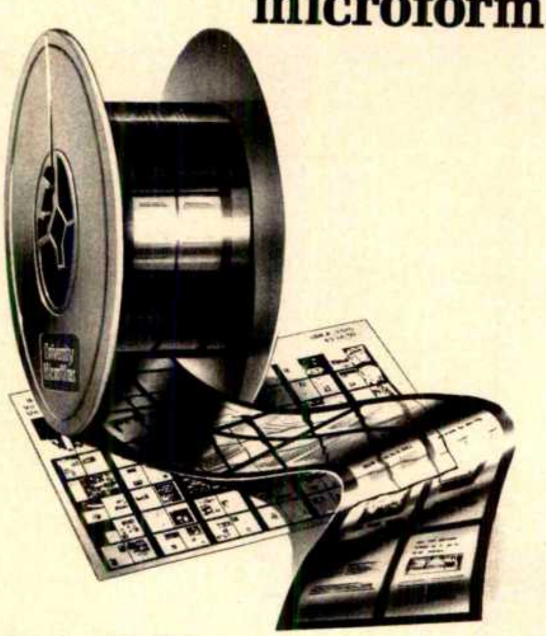

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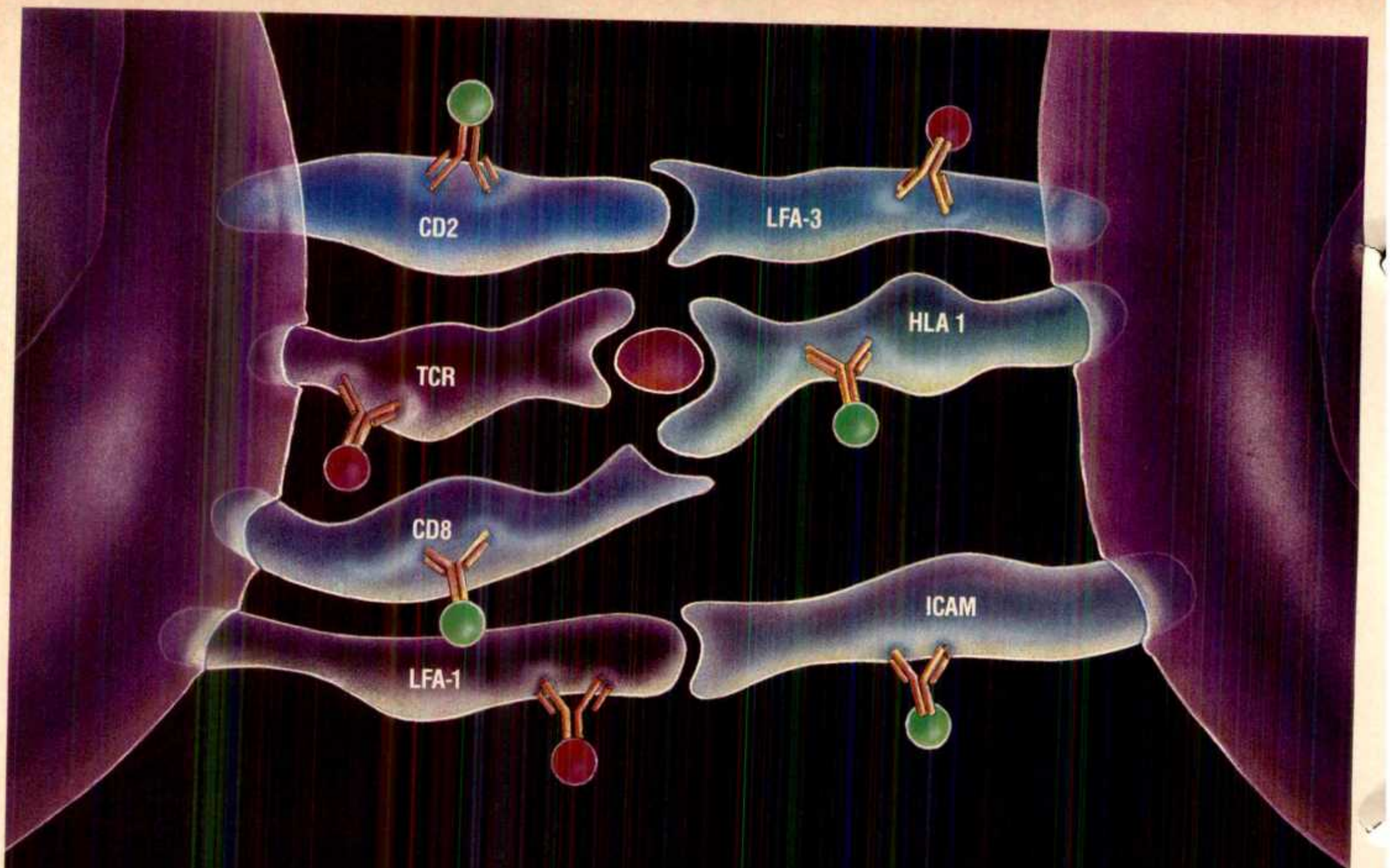

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Reader Service No 329

Antibody-directed drug discovery

M.E. Wolff and A. McPherson

By harnessing the remarkable power of the immune system to access the three-dimensional chemical information sequestered in target sites for drug ligands, antibody-directed drug discovery speeds the design of new therapeutic entities.

FOR more than a century, the search[1] for new drugs has been rooted in an organic chemistry-driven technology based on the synthesis and biological evaluation of large numbers of candidate compounds. Highlighting the inefficiency of this process is the estimate in *Bio/Technology* that US pharmaceutical companies spent about $14,000 million in 1989 to achieve approvals of only 22 new chemicals. On average, 10,000 candidates were prepared and tested for each successful drug.

The growing costliness and lengthiness of traditional methodology has focused renewed attention on what has been called rational drug design — the ability to create pharmaceuticals on the basis of a detailed knowledge of their structure and their interaction with biological targets. Today, most major pharmaceutical companies and several start-up companies have organized efforts in rational drug design. Several distinct strategies are used, including approaches based on computer modelling[2] of data obtained from binding assays and enzyme crystallography, as well as methods depending on bio-organic chemistry[3]. These applications of rational drug design thus require at least the purification and sometimes the crystallization of targets that are central to each disease process. Yet many prospective drug targets are membrane-bound macromolecules that cannot readily be crystallized or even identified.

Typical of the techniques being developed to tackle these difficulties are those of ImmunoPharmaceutics, under the term 'antibody-directed drug design' (Fig. 1). The remarkable power of the mammalian immune system is first harnessed to access the three-dimensional structural information contained in pharmacological receptors, enzymes and viruses without the need for special purification or isolation efforts. Then, the pharmacophoric information embedded in the resulting monoclonal antibody is read out by one of several methods and finally used to construct a peptidomimetic drug. These steps are considered briefly in the next sections.

Antibodies to drug targets

Through monoclonal technology, idiotypic antibodies (Ab-1) to key drug action sites in receptors, enzymes and viruses can be selected and produced in any desired amount without the need to isolate the target (Fig. 2). The idiotypic antibody is a mirror image of the target site, both in a spatial and electronic sense. By using the idiotypic antibody as an antigen, an anti-idiotypic antibody (Ab-2)[4], can be produced as a positive image of the target site (see Fig. 2).

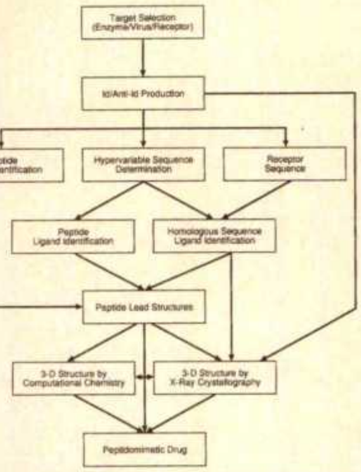

Target Selection (Enzyme/Virus/Receptor)
Id/Anti-Id Production
Peptide Ligand Identification
Hypervariable Sequence Determination
Receptor Sequence
Peptide Ligand Identification
Homologous Sequence Ligand Identification
Peptide Lead Structures
3-D Structure by Computational Chemistry
3-D Structure by X-Ray Crystallography
Peptidomimetic Drug

FIG. 1 Technologies for antibody-directed drug design.

Important drug design information is obtained from such antibodies or their fragments or complexes by crystallization using conventional protein crystallization techniques[5]. After collection of X-ray diffraction data the crystal structure is solved using the conventional multiple isomorphous replacement technique requiring heavy atom derivitization of the crystals. An alternative approach is that of molecular replacement, which depends on some degree of knowledge of the structure of the antibody, the antigen, or both.

As an example, the three-dimensional structure[6] of an antilysozyme Fab-lysozyme complex is shown in Fig. 3. A close-up view (Fig. 4) shows the spatial and electronic complementarity of the interacting surfaces of lysozome residues 41–46 and light-chain residues 55–59 that contributes to the hydrophobic, electrostatic, and hydrogen bonding accounting for the nanomolar affinity of the two ligands.

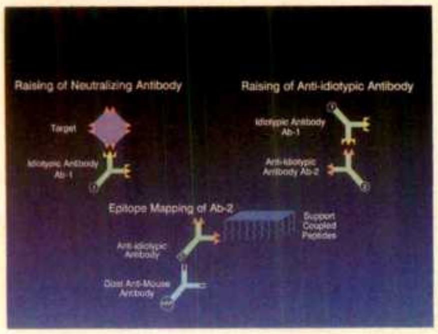


FIG. 2 Initial steps in antibody-directed drug design.

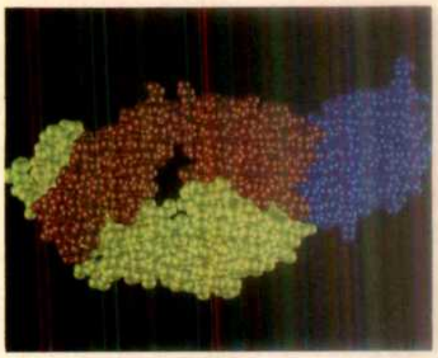


FIG. 3 Monocolour-coded CPK model representation of lysozyme-HyHEL-5 complex[6] obtained by Drs V.N. Balaji and U.C. Singh on an Evans and Sutherland PS 390 graphics system using MOGLI graphics display software and coordinates in the Protein Data Bank. Blue atoms: lysozyme; red atoms: light chain; and yellow atoms: heavy chain.

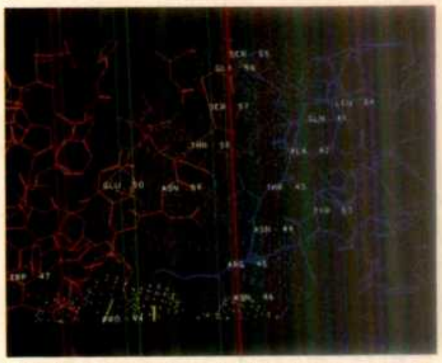


FIG. 4 Monocolour-coded stick model close-up view of the contact area of Fig. 3. Contact residues (<4 Å) are highlighted and shown as solvent-accessible surface.

These specific interactions between antibody and antigen demonstrate dramatically the potential of the approach for directed drug design. Such analyses provide an unequalled structural basis for the the creation of synthetic molecules, peptides, or peptidomimetics that share with the idiotypic antibody the ability to interact with the antigen. Related information can be deduced for anti-idiotypic antibodies that are replicas of receptors or other inaccesible macromolecules.

Extraction of information

In addition to X-ray crystallography, three other techniques (see Fig. 1) are used to read out structural information from the embedded pharmacophore in the antibody or from the anti-idiotypic antibody. By using the polymerase chain reaction, the amino acid sequence of the hypervariable loops of the antibodies can be determined. Alternatively, peptides that are complementary to the surface of anti-idiotypic receptor surrogates can be synthesized using solid-phase methods (see Fig. 2)[7]. A properly selected peptide that is complementary to a receptor surrogate results in a receptor antagonist. Lastly, evidence for the location of key amino acid sequences in the hypervariable loops of anti-idiotypic antibodies can be adduced from their homology with sequences in the initial antigen[8].

Peptidomimetic synthesis

The preceding process results in the design of a peptide drug that may be an enzyme inhibitor, a receptor antagonist (or sometimes an agonist), or an antiviral. Because peptides typically have short biological half-lives and poor oral bioavailability, it remains to synthesize a peptidomimetic[9] based on the peptide structure that is biologically active and can be administered orally.

The design of such peptidomimetics requires two different applications of computer-assisted drug design (CADD) techniques (V. N. Balaji and U. C. Singh, personal communication). First, if an estimate of the bioactive conformation of the peptide is not available from X-ray or nuclear magnetic resonance, data must be obtained from primary sequence information through the application of computational chemistry algorithms[10,11]. Based on the predicted three-dimensional spatial and electronic characteristics of the peptide, a proprietary database and algorithm of peptidomimetic surrogate elements is used to design candidate drugs for synthesis. Docking routines may be used to optimize complementarity in those cases where the three-dimensional structure of the receptor surrogate has been obtained[10,11].

Antibody-directed drug design offers wholly new insights and approaches in the design and optimization of new compounds for therapeutic application, and risk is minimized by the multiplicity of available strategies. As these methods are developed further, even greater savings in time and effort will be at hand in the search for new drugs. ☐

Manfred E. Wolff is Vice President for Research and Development, ImmunoPharmaceutics, Inc., 11011 Via Frontera, San Diego, California 92127, USA, and Alexander McPherson is Professor and Chairman of the Department of Biochemistry, University of California, Riverside, USA. For more information, fill in reader service number 100.

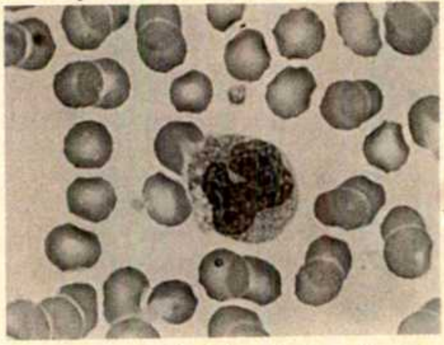
Immunology roundu

A colour processing system for gold-tagged antibodies, a tumour necrosis factor α [^{125}I] RIA kit, and a whole host of monoclonal antibodies are among this week's new products for immunological research.

As a follow up to its current line of human MAPPing (message amplification phenotyping) Amplimers, Clontech has issued new mouse cytokine MAPPing Amplimers for **cytokine analysis** using the GeneAmp PCR process (*Reader Service No. 101*). Designed to be faster and more sensitive than conventional methods such as immunoassays, and without using radioactivity, the MAPPing process involves the isolation and reverse transcription of mRNA and the specific PCR amplification of cytokine cDNAs. Each MAPPing amplimer set contains a pair of primers for a specific cytokine and a detailed protocol. Amplimers are sold under licence from Perkin-Elmer Cetus and produced in collaboration with Genelabs.

Eastman Kodak has developed a complete **colour processing system** — the Gold Plus enhanced immunogold staining kit — for the visualization by light microscopy of gold-tagged biological specimens (*Reader Service No. 102*). First, specific antigens are localized with gold-tagged antibodies. Then, silver halide is reduced in the presence of hydroquinone and adsorbed onto the colloidal gold surface, amplifying the signal. Following the silver-enhancement step, the specimens are placed in a stop bath and fixed. The biological film emulsion is then dip coated using the Gold Plus reagents: cyan, magenta or yellow. The colour processing system uses the silver-enhanced gold tag as the catalytic centre for the colour coupler/dye reaction, with colour development taking 3 to 60 seconds, says Kodak. Processing options are available further to enhance the colour intensity in accordance with the signal desired and the sample background. Colour signals are particularly useful when working with thick tissue sections and with structures having high inherent density. Each Gold Plus kit contains sufficient reagents for 50 preparations.

For the **one-step separation of monocytes** from leucocyte-rich plasma and whole blood, Nycomed recommends its specially formulated Nycodenz monocytes solution (*Reader Service No. 103*). Traditional methods of monocyte purification rely on the adherence properties of cells and, consequently, a significant proportion of cells are discarded, says Nycomed. Nycodenz can be used to isolate all classes of monocytes. The solution provided is slightly hypertonic: this causes shrinkage of lymphocytes, which pellet to the bottom of the tube while the monocytes remain at the top of the gradient. Full separation is achieved by overlaying Nycodenz with whole blood and centrifuging at 600 *g* for 15 minutes. Nycodenz is supplied sterile and in ready-to-use 100-ml quantities. When kept sterile and protected from exposure to light, the solution is stable for up to five years.



For the one-step isolation of monocytes, use Nycodenz from Nycomed.

Promega has a new complement of **extracellular matrix products** that should be of particular interest to cell culture researchers (*Reader Service No. 104*). E-C-L cell attachment matrix, which is rich in enactin, laminin and collagen type IV, promotes the attachment and spreading of many anchorage-dependent cell types, says Promega. It is designed either to coat culture substrates or be incorporated into culture media. On the other hand, human fibronectin, Promega's plasma-derived protein, can be used in cell culture to augment the attachment of a variety of cell types under reduced or serum-free conditions. Human fibronectin has high purity and is virally inactivated, says Promega.

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1. Burger, A. in *Burger's Medicinal Chemistry* 4th Edn, Pt 1 (Ed. Wolff, M.E.) 1–54 (Wiley, New York, 1980).
2. DesJarlais, R.L., Sheridan, R.P., Seibel, G.L., Dixon, J.S. & Kuntz, I.D. *J. med. Chem.* **31**, 722–729 (1988).
3. Walsh, C.T. *Trends biochem. Sci.* **8**, 254–257 (1983).
4. *Anti-idiotypes, Receptors, and Molecular Mimicry* (Eds Linthicum, D.S. & Farid, N.R.) 1–154, 255–284 (Springer, New York, 1988).
5. McPherson, A. in *The Preparation and Analysis of Protein Crystals* (Wiley, New York, 1982).
6. Sheriff, S. *et al Proc. natn. Acad. Sci. U.S.A.* **84**, 8075–8079 (1987).
7. Geysen, H.M., Rodda, S.J., Mason, T.J., Tribbik, G. & Schoofs, P.G. *J. immunol. Meth.* **102**, 259–274 (1987).
8. Williams, W.V. *et al Proc. natn. Acad. Sci. U.S.A* **85**, 6488–6492 (1988).
9. Goodman, M. *Bioponymers* **24**, 137–155 (1987).
10. Kuntz, I.D. *Protein Eng. (England)* **1**, 147–148 (1987).
11. Cohen, F.E., Gregoret, L., Presnell, S.R. & Kuntz, I.D. *Prog. clin. biol. Res.* **289**, 75–85 (1989).

Lastly, Promega's rabbit, anti-human fibronectin can be used to localize fibronectin by immunological means.

Metachem Diagnostics is the UK distributor of Advanced Magnetics' **tumour necrosis factor α (TNF α) [^{125}I] RIA kit**, which can be used to measure levels of natural and recombinant TNF α in serum, citrated or heparinized plasma samples, or cell culture supernatants (*Reader Service No. 105*). The solution-phase RIA is based on the 'sandwich' principle and uses an ^{125}I-labelled monoclonal antibody (mAb) and a mAb that binds to TNF α as well as to the solid phase. Incubation of TNF α with the two mAbs results in the formation of a complex where TNF α is sandwiched between the antibodies. The complex is separated from solution by magnetic separation or centrifugation with magnetic second antibody, which binds the non-isotopic monoclonal portion of the complex. The magnetic pellet that forms is counted in a gamma counter and the TNF α concentration determined by interpolation from a standard curve. The RIA has a sensitivity of 3.08 pg 0.1 ml^{-1}, is highly specific, and has negligible cross reactivity, says Metachem. Each kit contains sufficient reagents for 100 sample tubes including standard curves.

Cytokines: Il-8

The latest cytokine from the Bachem stable is **endothelial interleukin-8** (Il-8), a 77 amino acid variant of monocyte Il-8 with a pentapeptide extension at the amino terminus (*Reader Service No. 106*). According to Bachem, endothelial Il-8 has been shown to promote neutrophil chemotaxis and degranulation, and to inhibit neutrophil binding to endothelial cells in the presence of inflammatory stimuli. As the adhesion inhibitory activity helps to limit neutrophil-mediated endothelial damage during inflammation, Il-8 may provide new insights into natural anti-inflammatory mechanisms. Endothelial Il-8 is produced as a fusion protein in *Escherichia coli* and is >98 per cent pure by HPLC, says Bachem. It is fully active in chemotactic assays involving lymphocytes and neutrophils.

Boston-based Endogen has introduced a polyvalent **antibody to human interleukin-8** (Il-8) — the novel cytokine that has been shown to cause activation and chemotaxis of neutrophils *in vivo* (*Reader Service No. 107*). Anti-Il-8 is purified from the serum of rabbits immunized with the native sequence recombinant human cytokine. It is supplied sterile and free of carrier proteins and preservatives, says Endogen. Applications for the antibody include: use as a specific antibody reagent in ELISAs and radioimmunoassays; neutralization experiments; immunocytochemical staining; radioimmunoprecipitation; and immunoprecipitation in Western blots, immunoblots and slotblots. Endogen can also supply the Il-8 antibody coupled to a gel matrix. In this form, the precoupled antibody is supplied complete with buffers and a reusable column, and is suitable for use in cytokine purification, immunoprecipitation and neutralization procedures.

The cytokine **interleukin-8** (Il-8) and the antibody anti-Il-8 are two new products from Janssen Biochimica (*Reader Service No. 108*). Il-8, which is produced by monocytes, fibroblasts, T cells, endothelial cells, dendritic cells and keratinocytes, is known to stimulate neutrophil and T-cell chemotaxis, activate leucocytes and augment neutrophil lysosomal enzyme release, superoxide generation and antibacterial and antifungal activities. In excessive concentrations, Il-8 can cause plasma leakage and can increase vascular permeability. In addition, high levels of Il-8 are associated with pathological states — psoriasis, rheumatoid arthritis and asthma. Completing the picture, is Janssen's polyclonal rabbit, anti-human Il-8, which is specific to Il-8, does not cross react, and recognizes both natural and recombinant forms, says Janssen. Each vial of anti-Il-8 contains 1 mg ml^{-1}, and is free of preservatives and carrier proteins.

Il-8 and rabbit, anti-Il-8: now available from one source — Janssen.

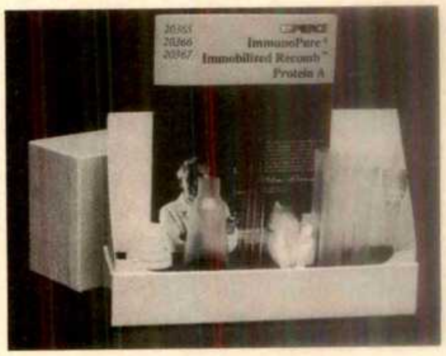
Cytokines: Il-2

Pharmacia Diagnostics' **natural, human interleukin-2** (Il-2) is new to the marketplace and is designed to provide an economical alternative to producing, purifying and testing Il-2 in the laboratory (*Reader Service No. 109*). Natural Il-2 stimulates the growth of a variety of T-cell types and clones and is suitable for both human and murine studies, says Pharmacia. Each lot of natural Il-2 has a minimum potency of 640 units per ml and is stable for an indefinite period when stored at −20 °C. Pharmacia's Il-2 is supplied sterile in ready-to-use 10- and 50-ml quantities complete with data sheet.

Dynal has developed a rapid **bioasay for Il-2** that does not require the maintenance of Il-2-dependent cell lines (*Reader Service No. 110*). The assay uses Dynabead M-450 magnetizable polymer particles coated with anti-CD3 to isolate T cells that are activated to express Il-2. CD3-positive T cells isolated in this way are, says Dynal, free from accessory cells and, as such, express Il-2 receptors but do not produce Il-2. The presence of extrogeneous Il-2 causes these cells to exhibit a proliferative response, which is Il-2 dose-dependent and can be measured by the incorporation of tritium-labelled thymidine. The assay has a sensitivity of 0.01 U Il-2 ml^{-1}, which is comparable to existing methods that rely on cells obtained from long-term culture. It has a dose-response curve that is linear up to at least 20 U Il-2 ml^{-1}, says Dynal.

Protein binding

Pierce now offers **immobilized recombinant Protein A** with a leak-resistant linkage (*Reader Service No. 111*). This recombinant form of Protein A immobilized on a cross-linked agarose matrix prevents the mitogenic effects that can occur with native Protein A contamination of samples, says Pierce. The recombinant Protein A is harvested from a non-pathogenic form of Bacillus that has been genetically engineered to secrete the protein. Consequently, recombinant Protein A is enterotoxin-free. Immobilized recombinant Protein A is compatible with Pierce's ImmunoPure (A) IgG buffer system and its gentle Ag/Ab buffer system.



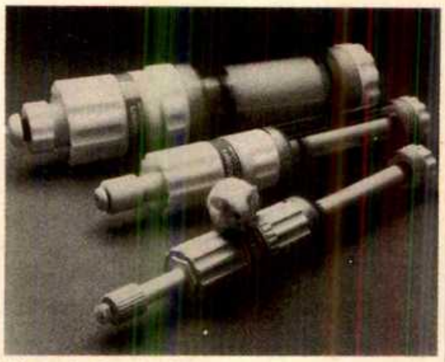
Pierce's immobilized Protein A with leak-resistant linkage.

Genex has eight new GammaBind **Protein G conjugates** designed to provide increased sensitivity and lower background interference with Western blots or ELISAs (*Reader Service No. 112*). GammaBind G is a genetically engineered Protein G that binds only IgG from a broad range of mammals, including all subclasses of human, mouse, rat, goat, sheep, cow, horse and rabbit. Genex has made available GammaBind G-gold for blotting purposes and GammaBind G-HRP, FITC and alkaline phosphatase for ELISAs. Completing the line up is GammaBind G biotin with three streptavidin conjugates for ELISAs and immunohistological studies.

Pre-bound recombinant **Protein G antibody purification devices** are now available from Nalge (*Reader Service No. 113*). Protein G has been shown to be useful for immunoglobulin purification, especially with murine monoclonal antibodies. It has a higher selectivity than Protein A for human, mouse or other species' classes of IgG and shows no cross reactivity with IgM, IgE, IgA or IgD, says Nalge. Scale up is made possible by stacking multiple 25- or 47-mm matrix discs in a holder, or by using Nalge's pre-stacked 50-mm units. Typically, recombinant Protein G-bound 50-mm units have a maximum yield of 16 mg of human IgG.

Pre-bound rProtein G antibody purification devices from Nalge.

Protein-Pak HR high-performance **ion exchange glass columns** from Waters are designed for the analytical and preparative separation of immunoglobulins from ascites or cell culture supernatants (*Reader Service No. 114*). Packed with 8-, 15- or 40-μm particle size anion (DEAE) or cation (SP) exchange resins (1,000 Å), the Protein-Pak HR columns provide improved resolution, protein binding capacity and recovery, says Waters. Scale up is simplified by the use of different column dimensions: 10, 20, or 50 mm × 100 mm. When used in combination with Waters' 650 advanced protein purification system, performance of the Protein-Pak columns is optimized.


Waters' Protein-Pak ion exchange glass columns come pre-packed.

Isotyping kits

The new monoclonal antibody (mAb) panel from Silenus Laboratories of Australia provides researchers with a direct method of **serotyping *Neisseria gonorrhoeae*** (*Reader Service No. 115*). The ten mAbs react specifically with the epitopes of protein I of *N. gonorrhoeae* and can be used for the direct detection of *N. gonorrhoeae* in patient specimens, or for colony confirmation or research applications. Silenus sells the mAbs individually or as a cocktail of ten mAbs that will react with 98 per cent of serovars worldwide. In both forms, they can be supplied either unconjugated or conjugated with FITC.

With the INNO-LIA mouse monoclonal antibody (mAb) **isotyping kit**, all murine subclasses — IgG1, IgG2a, IgG2b, IgG3, IgM, IgA and IgE — can be detected in less than one hour, says Belgium-based Innogenetics (*Reader Service No. 116*). The protocol differs from conventional ELISA techniques in that rat mAbs against the different subclasses are applied in fine lines to a membrane-coated strip. One test strip is used per sample. Using monoclonals for the purposes of detection eliminates problems of cross reactivity, says Innogenetics, and provides a sensitivity of 0.1 μg ml^{-1}. Results are read visually and the strips can be stored as a permanent record.

Innogenetics' INNO-LIA mouse isotyping kit for speed and sensitivity.

Oncoproteins

Adding to its list of over 2,000 immunological reagents, Bioproducts for Science has introduced a new line of **oncoproteins and antibodies to oncoproteins** (*Reader Service No. 117*). Oncoprotein antibodies are site-specific probes raised against small synthetic peptides that are selected from a particular oncoprotein's amino acid sequence. They can be used to recognize identical sequences in intact native proteins as well as the synthetic peptide. The antibodies have been characterized against both the synthetic peptide and the native protein released from cells expressing the relevant oncoprotein, says BPS, and are supplied as purified immunoglobulin complete with test data and recommended working dilutions. Stated applications for the antibodies include immunoprecipitation, immunoblotting, immunohistochemistry, affinity purification and neutralization studies.

Capitalizing on Cambridge Research Biochemicals' expertise in the synthetic peptide approach to antibody production, the company offers a range of **murine hybridomas secreting antibodies** capable of specifically targeting epitopes contained within the cytoplasmic and external domains of both human epidermal growth factor-R (EGF-R) and c-*erb*B-2, and from the nucleus-associated *myc* oncoprotein family (*Reader Service No. 118*). All have been characterized for native protein reactivity by immunoblotting, flow cytometry, immunoprecipitation and immunocytochemistry. In view of the importance of reagents specific for EGF-R, c-*erb*B-2 and *myc* proteins in the diagnostic and prognostic assessment of various abnormal physiological states, CRB's antibodies may have considerable value in clinical, diagnostic and therapeutic research. The hybridomas are available for licensing or purchase.

Monoclonal assortment

The latest addition to BioGenex's panel of antibodies for the identification of lymphoma and leucocyte markers on paraffin-embedded tissues includes a **monoclonal antibody to human macrophage** — LN-5 (*Reader Service No. 119*). LN-5 stains the cytoplasm of macrophages and histiocytes in haematopoietic organs and their derived tumours, says BioGenex. It is strongly reactive with cases of true histiocytic lymphoma, but negative, except in macrophages, in Hodgkins and non-Hodgkins lymphomas. LN-5 is available as a ready-to-use reagent, or as a complete kit with the StrAviGen super-sensitive detection system. The kit provides sufficient reagents for up to 50 slides.

Identi-T CγM1 is T Cell Sciences' latest **monoclonal antibody to the human T-cell antigen receptor** (TCR) (*Reader Service No. 120*). The mAb identifies a framework (or common) determinant of the γ chain of the human γδ TCR. The determinant can be detected on fixed cells and frozen tissue sections, but is not intended for flow cytometry analysis as the binding determinant is not exposed on the surface of live cells, says T Cell Sciences. Identi-T CγM1 has applications in immunohistology protocols, immunoprecipitation procedures and Western blot determinations to investigate the role of T lymphocytes in lymphoid malignancies, autoimmune diseases and immune system disorders. The antibody is available in a ready-to-use purified form — sufficient for 100 tests.

The monoclonal antibody **anti-mouse CD3-ε**, an important tool in the immunologist's armoury for cellular studies involving the mouse model, is now available from Boehringer Mannheim (*Reader Service No. 121*). The anti-mouse CD3-ε, is a marker for targeting mature mouse T cells expressing the T-cell receptor. It can also be used directly to activate T cells, independent of antigen binding. The antibody is supplied in a purified form and is function tested by flow cytometry. For simple, one-step labelling techniques, anti-mouse CD3-ε is available conjugated to fluorescein.

Anti CD3 - Mouse Monoclonal Antibody
Contains 0.1% sodium
For Research Use Only
Not for use in diagnostic
Mouse - Fluorescein Monoclonal Antibody
Contains 0.1% sodium
For Research Use Only
Not for use in diagnostic
Protect from light

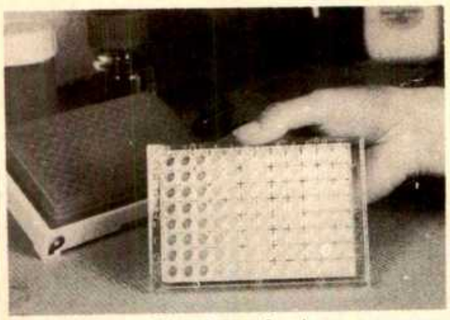
Boehringer's anti-mouse CD3-ε simplifies T-cell mouse studies.

New in the immunological marketplace is Serotec's **mouse anti-rat natural killer (NK) cell monoclonal antibody**, which recognizes and reacts with all rat NK cells, large granular lymphocytes and neutrophils (*Reader Service No. 122*). The antibody is available in three forms: 5-ml vials of culture supernatant containing 5 μg ml^{-1}; 0.25-ml vials of ascites containing 1–2 mg ml^{-1}; and as a F(ab$'$)$_2$ FITC-conjugate in 1-ml vials containing 700 μg ml^{-1} of antibody.

Serotec's mouse anti-rat natural killer cell monoclonal antibody.

Everything for ELISAs/RIAs

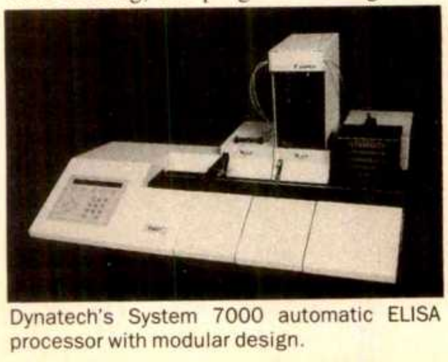
Polyfiltronics has a new line of **microtitre plates** called Filtaplates, which have 96 open-bottomed wells, each of which is sealed with a membrane that acts as a solid-phase support (*Reader Service No. 123*). Filtaplates can be equipped with membranes of various pore sizes and characteristics: high protein binding membranes for immunoassays, and low protein binding membranes for filtration purposes. According to Polyfiltronics, the patented encapsulation system of the plate eliminates cross-talk between wells. Liquid flowthrough can be augmented by using the Filtaplate in conjunction with Polyfiltronics' vacuum manifold system. The company also supplies activated 96-well microplates that have been chemically treated for the covalent attachment of proteins.



Polyfiltronics' Filtaplates for immunoassays and filtration purposes.

Wellfill 4 from Denley Instruments functions as both a single- and multi-reagent **dispenser for microtitre plates** (*Reader Service No. 124*). Suitable for enzyme-based or radioimmunoassays, Wellfill 4 can dispense up to eight different reagents in the range of 10 to 400 μl. Fixed volumes for routine applications are selected with plug-in cards. In addition, a fully programmable card is available for user control of dispense volumes that can be set to dispense in 1-μl increments. All parts of the Wellfill 4 dispenser that are in contact with liquid can be removed and autoclaved.

Dynatech now offers an **automatic ELISA processor** — the System 7000 — that is based on the company's MR 7000 automatic microplate reader (*Reader Service No. 125*). When functioning as a stand-alone automatic reader, the MR 7000 can read a plate in about 1.7 seconds, says Dynatech. Users can also choose from several optional features, including temperature control, UV operation, bar-code reading, and program cartridges for agglutination reading, kinetic analysis and tissue culture reading applications. By virtue of its modular design, the MR 7000 can be expanded to give full ELISA processing potential. Various add-on modules are available for microplate washing, dispensing and diluting, and random-access plate storage. The systems's advanced plate transport system allows the single-plate carrier to travel from the reader to any number of the add-on modules up to a distance of two metres.



Dynatech's System 7000 automatic ELISA processor with modular design.

Newsbeat

Take the effort out of antibody and immunoassay development with Berkeley Antibody Company's (BAbCO's) **custom antibody and immunoassay development services** (*Reader Service No. 126*). BAbCO will generate polyclonal or monoclonal antibodies against peptides, proteins, organic haptens and other molecules to order. Also available are custom antigen preparation services for isolating, purifying, and/or conjugating your antigen. The line-up of other services includes: polyclonal antisera production in small and large animals, custom hybridoma development, scale-up of mAb production in ascites fluid, and immunobiochemical procedures that include antibody conjugations, purifications, fragmentation, biotinylation, isotyping, enzyme and fluorescent labelling. BAbCO also provides immunoassay development services using ELISA, PCFIA and Western blot formats.

BAbCO's catalogue of custom antibody and immunoassay development.

Materials and services pertinent to cell physiology, virology, oncology and immunology can be found in the **1990 catalogue** from Lee and BioMolecular (*Reader Service No. 127*). The extensive listing includes interferons, animal viruses, antiviral sera and immunoglobulins. Many of the new products are particularly applicable to the development of vaccines and diagnostic products. Also outlined are research services for the identification and quantification of viruses, interferons, anti-interferon and antiviral

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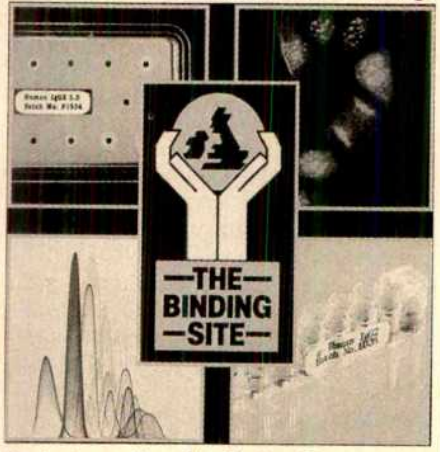
WANTED
Ideas for Software Development
CHEMISTRY BIOCHEMISTRY
BIOTECHNOLOGY MEDICINE

I develop and distribute software for the above mentioned fields. I am searching for partner who can contribute product ideas and know-how. They have no further obligations and can participate on profit. If wished, a further going engagement is possible.
I have experience with Realtime-UNIX (QNX), HPUX, DOS, UNIFY and C.
M. Weidmann — Technische EDV-Systeme
Oberhofstrasse 41, CH–6020 Emmenbruecke, Switzerland
Telephone: 041-559-329 Fax: 041-559-329

Reader Service No.21

immunoglobulins, sterilization of biologicals and *in vitro* toxicity testing.

More than 300 new immunological reagents and kits for use in research, clinical and diagnostic applications are featured in the latest **catalogue** from The Binding Site (*Reader Service No. 128*). The new product listings include IgG subclass reagents in kit form for the nephelometric assay of subclass levels, and a range of purified myeloma proteins; five EIA kits for the investigation of functional IgG activity; and antisera and conjugates to mouse and rat proteins of a higher titre than previously offered.



Product news from The Binding Site.

Outlined in the FisherBiotech **ELISA systems brochure** are a series of microplate readers, providing varying levels of automation to suit laboratory needs (*Reader Service No. 129*). A range of equipment and supplies are featured in the brochure: from kinetic microplate readers, automated washers and software to immunological accessories such as microplates and reagents. Details of the latest ELISA data management system are included, which combines an IBM PS/2 PC with Fisher's Kineti-Calc software and provides full instrument control and data handling capabilities.

Pierce and Warriner says its 340-page full colour **immunotechnology catalogue and handbook** features over 200 new immunochemical products (*Reader Service No. 130*). This latest catalogue includes product information, protocols and references on the following subject areas: immunogens, antigen/antibody purification, antibodies, avidin-biotin, and protein modification.

Immunologists can now obtain the 1990 Jackson **immunoresearch catalogue** from Stratech Scientific (*Reader Service No. 131*). New product lines include affinity-purified antibodies and proteins, a range of phycoerythrin conjugates, Affinipure antibodies for double or multiple labelling and AMCA conjugates.

Immunoreagents/assays

Novabiochem recommends Liposorb for the **selective removal of lipoproteins** from blood plasma or serum, while allowing the antibody content of antiserum or the coagulation factors to remain in tact (*Reader Service No. 132*). Liposorb can be used as a dry substance, in batch procedures or by column. Novabiochem says Liposorb has low solubility in solvents, providing, says Novabiochem, stability in acids and bases. Liposorb is thermally stable at high temperatures and can, therefore, be sterilized by autoclaving.

Putting safety first, Day Impex announces that its **virucidal disinfectant**, Virkon, is effective against all 17 known virus families, including HIV-1 and hepatitis A or B (*Reader Service No. 133*). This broad-spectrum disinfectant is also an effective bactericide and fungicide. In a single operation, users can disinfect work-surfaces and equipment with Virkon, which is water-soluble, almost odourless, non-corrosive, non-tainting and of a very low toxicity, says Day Impex. Virkon is supplied in powdered form and can be applied directly to spills of blood or body fluids.

According to Life Technologies, cryopreserving a broad spectrum of mammalian cell types need not be a problem with its ready-to-use **cell culture freezing media** (*Reader Service No. 134*). The media are available in two formulations: one with dimethylsulphoxide, the other with glycerol. Both are prepared using Dulbecco's modified Eagle medium. To ensure high viability and sustained cell growth on thawing, fetal bovine and calf sera supplements are used. The media are quality-control tested with both adherent and suspension cells.

Using radioimmunoassay technology, Amersham's healthcare and life science division has introduced a **[^{3}H] scintillation proximity assay (SPA) for platelet activating factor** (PAF) (*Reader Service No. 135*). The assay affords researchers with a reliable and precise means of measuring PAF in biological samples, says Amersham. The SPA technology requires no separation of bound from free liquid and no addition of liquid scintillants. Using a highly specific PAF antibody and a high specific activity tritiated PAF tracer, PAF can be measured in the range of 20–1,280 pg per tube, says Amersham. Each pack contains sufficient reagents for 100 assay tubes. ☐

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

Nature Classified

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF. Telephone: 071 872 0102. Fax: 071 240 2408.
NEW YORK – Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone: (212) 477 9625. Fax: (212) 505 1364.
PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 40 53 03 39. Fax: (1) 42 67 64 99.
MUNICH – Sabine Fürst, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 267 8751. Fax: (03) 267 8746.

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THE PETROLEUM SCIENCE AND TECHNOLOGY INSTITUTE
EDINBURGH AND HERIOT-WATT UNIVERSITIES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a three-year appointment as a geochemist to join a research group directed by Professor Brian Smart, and Drs. Ian Main, Graham Shimmield and Stephen Elphick. The project entitled "The Effects of Combined Changes in Pore Fluid Chemistry and Stress State on Reservoir Permeability", forms part of the core research programme of the recently founded Petroleum Science and Technology Institute. Using state-of-the-art analytical and rock mechanics technology, a series of rigs to simulate *in situ* petroleum reservoir conditions will be constructed.

The successful candidate will be employed on the geochemical aspects of this study in close collaboration with Dr Graham Shimmield. Experience of analytical and inorganic chemistry is essential; knowledge of HPLC, IC and FIA techniques would be advantageous. Specific responsibilities will include the establishment of on-line geochemical analysis of changes in pore fluid chemistry under applied stresses. Rock fabric studies will be carried out with ion probe and SEM techniques for which appropriate training will be given.

The salary will be on the research staff grade 1A (£10,458 to £16,665) depending on age and experience. Applicants should submit their c.v. and the names of two referees to Mrs Patricia Stewart, Secretary to the Petroleum Science and Technology Institute, Grant Institute, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JW, by the 6th June, 1990. PLEASE QUOTE REFERENCE NO. 5773.

(3649)A

Jessie Boden Lloyd Professorship in Immunology Research

The University of Calgary Faculty of Medicine

invites applications for the Jessie Boden Lloyd Professorship in Immunology Research. This full-time academic position at the **Associate Professor** level or higher will be associated with the Immunological Sciences Research Group, a multidisciplinary group of ten faculty. Preference will be given to senior, well-established researchers in immunology, immunophysiology, or neuroimmunology, and especially to candidates with interest in mucosal or articulating surfaces. Opportunities exist for additional recruitment externally funded through provincial and national agencies.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary has an Employment Equity Program and encourages applications from all qualified candidates, including women, aboriginal people, visible minorities, and people with disabilities.

Interested candidates should submit a curriculum vitae, the names of three referees, and a statement of research interests and goals by June 30, 1990, to:

Dr. M. Watanabe, Chair, Advisory Committee, Jessie Boden Lloyd Professorship, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1.

(NW5342)A

CELL BIOLOGIST

Pharmaceutical Research

At Pfizer Central Research in Sandwich we are dedicated to the discovery and development of novel compounds for human and veterinary medicines. We have a major multi-million pound expansion of our facilities under construction and are looking to the future with confidence.

The current scientific emphasis within our Animal Health Research Group is on chemotherapy of parasitic diseases in pets and livestock and on agents to improve the performance of farm animals. To expand this programme we are seeking an enthusiastic scientist capable of leading a small team of biologists engaged in the application of cell-based approaches toward drug discovery. Candidates should possess a **PhD in Biochemistry or a biological subject**, and have experience in cell culture and enzyme or receptor assay techniques.

This is an excellent opportunity for you to influence the research direction of an important group within one of the world's leading pharmaceutical companies and provides an ideal environment for both scientific and professional development.

If you join us you will receive a highly competitive salary and bonus related to your own performance. Other benefits include flexible - working hours, pension scheme, private medical insurance and an active Sports and Social Club with first-class amenities. Naturally we will pay your removal expenses.

If you are self-motivated and seeking a challenge, we would like to hear from you. Please write, enclosing a CV to: Mrs Fiona Cox, Personnel Officer, Pfizer Central Research, Ramsgate Road, Sandwich, Kent. Telephone: 0304 618107.

Pfizer

(3700)A

Division of Wildlife and Ecology
National Rangelands Program
CANBERRA ACT
AUSTRALIA

RESEARCH SCIENTIST

Animal Production/Ecology

$A32,471 to $A39,724

THE DIVISION: The Division conducts research into the nature of Australia's ecological systems and their component species to provide a scientific basis for the management and conservation of wildlife, plant and land resources. It has a staff of 250 including 90 professional researchers. The headquarters for the Division is located in Canberra.

THE PROGRAM: The Division's National Rangelands Program is seeking a scientist to join a multi-disciplinary team researching the ecological principles underlying the development of more efficient and sustainable management systems in semi-arid rangelands. In particular the program is developing appropriate methodology for assessing when and where degradation occurs, ecologically based techniques for restoring degraded rangelands, efficient grazing management systems and decision support systems for technology transfer.

THE PROJECT: The appointee will:

(i) determine the productivity of sheep in semi-arid rangelands in relation to type and status of plant communities and forage supply,

(ii) determine the basis for and shape of relationships between sheep production parameters and competing native and feral herbivores at a property and regional level and

(iii) develop a property level sheep production model for rangelands and evaluate alternative stocking strategies.

THE PERSON: Applicants should hold a Ph.D. degree or equivalent qualifications and have a strong interest in animal production and/or animal/animal interactions. Whilst the person will be based in Canberra, it is important that they be prepared to conduct research in the field at remote locations, often under difficult conditions. An ability to work in a team is essential.

CONDITIONS: The position is for an indefinite term. Australian Government Superannuation benefits are available.

INFORMATION: Prospective applicants are invited to contact Dr Ken Hodgkinson for further information on 61 58 81 1133, or Fax 61 58 81 4185. A copy of the detailed duty statement and selection criteria may be obtained by telephoning Ms Brigid Malley on 61 6 242 1632. It is in the interests of applicants to obtain selection documents and frame their applications accordingly.

APPLICATIONS: Applications should be submitted by 31 July, 1990 and quote reference number PG90/18GU. They should be framed against the selection criteria, and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate at least two professional referees, and address their applications to:

The Chief
CSIRO Division of Wildlife
& Ecology
PO Box 84
LYNEHAM ACT 2602
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W7354)A ***AB1-PG90/18***

KING'S COLLEGE SCHOOL OF MEDICINE

Department of Clinical Biochemistry

Bessemer Road, London, SE5 9PJ

RESEARCH ASSISTANT

The project is to establish specific biochemical markers of bone turnover for the investigation of patients with osteoporosis and other metabolic bone diseases. The project will involve the use of HPLC and immunoassay techniques to identify possible marker compounds. The appointment will be made on the Research Assistant 1B scale and is for one year in the first instance with possible renewal for a further 1 – 2 years. The successful candidate would be encouraged to register for a higher degree.

Further details can be obtained from:

Rosamund Greensted — 071-326 3008.

Closing date for applications: 22nd June 1990. (3686)A

Biochemist or Molecular Biologist
Research Scientist

PEPTIDE TECHNOLOGY LIMITED/CSIRO

Peptide Technology Limited is a Sydney-based biotechnology company with research and production facilities in Sydney and Copenhagen (Carlbiotech A/S). In association with collaborating institutions both in Australia and in Europe it is developing novel therapeutic products for use both in human health-care and in animal production.

The Company requires an energetic and self-motivated scientist to study the significance of and prevention of free radical damage in skin cancer and aging in a joint project with CSIRO. The successful applicant will have a background in DNA or Protein Chemistry, Molecular or Cell Biology.

The successful applicant will work at CSIRO's Division of Biomolecular Engineering, North Ryde, Sydney in association with a number of other scientists working in similar or complementary fields.

An attractive salary will be negotiated depending on qualifications and experience. Other benefits including a generous superannuation scheme, share options and housing assistance is available.

Please write with full c.v. to the **General Manager, Peptide Technology Ltd. P.O. Box 444, Dee Why, NSW 2099, Australia or Fax to + 61 2 9828046.** (W7348)A

THE MAX-PLANCK-INSTITUTE FOR IMMUNOBIOLOGY
Freiburg

has available several postdoctoral positions to study mouse B cell development at the molecular and cellular level.

Send curriculum vitae with names of two references and a short summary of your research interest to:

Dr. Georges Köhler,
MPI für Immunbiologie,
Stübeweg 51, 7800
Freiburg/Germany.
FAX No. 0761-5108-221.

(W7347)A

PHD CRYSTALLOGRAPHER

There is a vacancy for a PhD Crystallographer with chemical/biochemical training and familiarity with database and expert systems at the Cambridge Crystallographer Data Centre. Strong mathematical and computing background is important, including VMS and UNIX systems expertise. Three year appointment subject to probationary period. Salary on Research Assistant/Associate scales. Please send C.V. and the names of 3 referees to **Dr. Olga Kennard FRS at the University Chemical Laboratories, Lensfield Road, Cambridge, CB2 1EW, UK.** (3661)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF PATHOLOGY

A vacancy exists for a technically qualified person to work in our new production facility. The duties will be directed towards the large scale production of immunodeficiency viruses and virus antigens for research purposes, the preparation of diagnostic tests and the development of experimental vaccines. The post is permanent, full time and available immediately.

Candidates should have a bi[illegible]logical science or relevant produ[illegible]tion based background and hav[illegible] a commitment to commercially orientated reserch and development. Expertise in either the fields of mammalian cell tissue culture technology or virology would be desirable but not essential.

The appointment will be made to either MTO or MLSO scales, the salary ranges being from £11,241-£14,792 per annum depending an age, qualifications and experience.

Further details and a job description can be obtained from Dr G H Farrar, telephone (0980) 610391, ext: 2560 or 2228.

National Health Service terms and conditions will apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Telephone (0980) 610391, ext 2240), to whom completed forms should be returned by 7th June 1990, quoting Post No. 1124. (3654)A

Cancer Research Campaign

SCIENTIFIC OFFICERS AND POST-DOCTORAL SCIENTIST

- We are the biggest funder of cancer research scientists, supporting some 700 projects in universities, medical schools and research institutes throughout Britain.
- We have developed a strong interface between research and clinical therapy, facilitating the effective development and implementation of cancer treatments.
- Of the nearly £40 million we raised through voluntary contributions in 1989, 92% went directly into research - accounting for around 40% of all cancer research in the UK.

THE BEATSON INSTITUTE FOR CANCER RESEARCH

This is a modern, well-equipped laboratory with 10 groups working in various areas of gene expression and tumour biology.

SCIENTIFIC OFFICER

A vacancy is immediately available within a Research Group studying nuclear oncogene function. Candidates should possess a B.Sc or equivalent in biological sciences. Experience in molecular biology would be an advantage but is not essential. Starting salary is in the range £9,679 to £12,956 per annum.

Applications with C.V. and the names of two referees should be sent to Dr.D. Gillespie at the address below.

SCIENTIFIC OFFICER

A vacancy is immediately available in the Papillomavirus Research Group headed by Dr. Campo. The successful candidate is expected to have a B.Sc, or equivalent degree. Experience in molecular biology and/or tissue culture techniques would be advantageous. Salary is in the range £9,679 to £12,956 per annum.

Applications with C.V. and the names of two referees should reach Dr. M. S. Campo by May 31st at the address below.

POST-DOCTORAL SCIENTIST

A post-doctoral position will be available from 1st October 1990 in Dr. P. R. Harrison's group which currently has interests in two main areas: selenoprotein gene expression in relation to the anti-carcinogenic effects of selenium; and genetic changes implicated in the abnormal proliferation and differentiation of leukaemic cells. Experience in molecular biology is essential. The post is available for three years in the first instance. The salary will be in the range of £13,527 to £16,665 per annum depending on age and experience.

Applications with CV and names of two referees should be sent to Dr. P.R. Harrison at the following address below.

THE BEATSON INSTITUTE FOR CANCER RESEARCH, GARSCUBE ESTATE, BEARSDEN ROAD, GLASGOW G61 1BD.
TEL: 041-942 9361. FAX 041-942 6521.

Fighting cancer on all fronts.

(3710)A

OPPORTUNITIES IN BIOTECHNOLOGY

Pharmaceutical Proteins Ltd is seeking ambitious research scientists and technicians in an expansion of its existing Molecular Biology team. The Company is dedicated to the production of therapeutically useful proteins in the milk of transgenic mammals and from October 1990, will be occupying new laboratories alongside the AFRC Animal Genome Centre in King's Buildings, University of Edinburgh.

We are looking for –

A Molecular Biology Team Leader

The ideal candidate would have a proven track record in the area of gene manipulation, and an ability to manage, motivate and organise a research team. Research responsibilities include the optimisation of foreign protein expression in the milk of transgenic mice, rabbits, sheep and cattle.

Molecular Biologists

Candidates will have PhD training in genetic manipulation and post-Doctoral experience is desirable though not essential. Research tasks include the construction of milk promoter-driven human genes and optimisation of their expression in transgenic animals and cell lines.

Research Technicians

Candidates will have had relevant training in molecular biology techniques. Duties will include DNA and RNA preparation and analysis.

The immediate financial rewards will be attractive and there will be opportunities to participate in the future success of the Company. Relocation is available in appropriate cases.

If you wish to be considered for any of these positions please contact:

Dr Ron James
Pharmaceutical Proteins Limited
Kings Buildings
West Mains Road
Edinburgh EH9 3JQ

Garvan Institute
Of Medical Research
St. Vincent's Hospital, Sydney, Australia

The Garvan Institute is the leading medical research institute in New South Wales and is affiliated with St. Vincent's Hospital and the University of NSW. The Institute is one of five recipients of block funding from the National Health and Medical Research Council and has a staff of 120 structured as 4 integrated divisions.

EXECUTIVE DIRECTOR/ PROFESSOR

The Board of the Institute invites applications from persons wishing to be considered for appointment as Executive Director of the Institute. The successful applicant will be appointed to an appropriate Chair in the Faculty of Medicine at the University of NSW and to the medical or scientific staff of St. Vincent's Hospital.

The new director should be an internationally recognised scientist and will be expected to lead the Institute by personal research, developing strategies for future development and by promoting the interests of the Institute in the scientific and public domains.

Further information may be obtained by contacting the Institute Secretary. Enquiries should be directed to the Chairman of the Board, Mr. Charles Curran, phone 61 2 257 0541.

Applications in writing, together with the names and addresses of at least three referees, should be forwarded by 24th July 1990 to:

THE SECRETARY
GARVAN INSTITUTE OF MEDICAL RESEARCH
ST. VINCENT'S HOSPITAL,
DARLINGHURST, NSW 2010 AUSTRALIA
TELEPHONE: 61 2 361 2050
FAX: 61 2 332 4876

(W7357)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

CANCER RESEARCH CAMPAIGN GROWTH FACTOR GROUP

Postdoctoral Research Assistant 1A

BR/331

The major focus of the CRC Growth Factor Group is the structural and functional analysis of growth and differentiation regulatory factors involved in early mammalian development. A combination of molecular biological, protein chemistry and experimental embryological approaches (including BS cell and microinjection mediated transgenesis are employed. Current project areas include; a) Functional characterisation and molecular cloning of novel differentiation regulatory factors. b) Experimental analysis of growth factor action by gene targeting and transgenesis techniques. c) Development of novel strategies for the identification and experimental manipulation of embryonic regulatory factors.

A postdoctoral position will become available from September 1990. Applications are particularly sought from individuals with a strong background in either recombinant DNA techniques or ES cells and experimental embryology who have an interest in bioregulatory factors. The positions are suported by the Cancer Research Campaign until January 1994 and, subject to review, may be renewed thereafter.

Salary on the scale £10,458 – £16,665 per annum, depending upon age and experience.

Informal enquiries may be directed to: **Dr John Heath, Telephone – (0865-275721). FAX – (0865-275259)**

Postdoctoral Research Assistant (RS-1A)

BR/330

A postdoctoral position is available in the Virus Molecular Biology Group, directed by Drs. Alan and Sue Kingsman. The research will involve a characterisation of the molecular mechanism of action of viral and cellular regulatory proteins.

Experience in one of the following areas would be an advantage: recombinant DNA techniques, cell biology, protein purification, transcription or splicing analysis. The position is for three years, and is available from October 1990, although an earlier start date is possible.

Applications should be sent by 21st June.

Salary on the scale: £10,458 – £16,665 per annum, depending upon age and experience.

Applications for both posts, including a full curriculum vitae, and the names and addresses of two referees, should be sent to: **The Adminstrator.**

An Equal Opportunity Employer (3685)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU

The Queen's University of Belfast

RESEARCH POST

Department of Pure and Applied Physics

The above post, funded by the Agriculture and Food Research Council, is available as soon as possible for three years, to carry out research into the dynamics of surface layers of protein and surfactant/protein mixtures using laser light scattering, conventional surface viscometry and fluorescence recovery after photobleaching. Applicants should preferably have a PhD in Physics or Chemistry or a good Honours Degree with at least 1-2 years relevant experience. Computing experience is desirable.

Salary scale: £10,458-£12,381 per annum, placing depending on qualifications and experience.

Applicants, quoting ref. 90/R1, should submit a curriculum vitae, including the names and addresses of two referess to the **Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland.** Closing date: 15 June 1990.

The University is an Equal Opportunity employer. The University may decide to interview only those applicants who appear, from the information available, to be the most suitable, in terms of experience, qualifications and other requirements of the post.

(3679)A

AFRC INSTITUTE FOR ANIMAL HEALTH

SENIOR MOLECULAR BIOLOGIST

UP TO £35,436

SENIOR MOLECULAR BIOLOGIST required to be involved in the co-ordination and management of 8 research groups in the areas of Microbial Genetics, Enteric viruses, Coronaviruses, Avipoxviruses, Avian Herpesviruses, Avian Genetics, Influenza and Molecular Parasitology. The postholder will also develop his/her own research group, preferably within one of these areas, and will assist the Head of Division with the management of staff and resources.

At present some of the laboratory groups are based at the Institute's Houghton Laboratory near Cambridge but on completion of new accommodation at Compton all work will be consolidated at the Compton site.

Qualifications: A PhD in an appropriate area of molecular science with considerable experience in the initiation and management of research programmes is essential for this post.

Salary: UG6 £23,039 to £30,001 with performance related increments up to £35,436.

Non-contributory superannuation scheme.

The post will be based at the Compton Laboratory and assistance with removal expenses up to £5,000 may be available.

Applications quoting 5/42/90 with curriculum vitae and the names of three referees to the **Personnel Officer, AFRC Institute for Animal Health, Compton, Newbury, Berkshire RG16 0NN.** For further details please telephone the Head of Molecular Biology, Dr. Chris Bostock, on 0635 578411.

An Equal Opportunities Employer (3683)A

GBF Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, FR Germany — a government supported Research Institute of Biotechnology — seeks a

Peptide chemist (postdoc)

The position is available immediately in the peptide synthesis laboratory of the Division of Cell Biology and Genetics. The successful candidates will be expected to participate in the development and improvement of novel automated solid phase synthesis, methods, in close collaboration with molecular biologists, immunologists and polymer chemists.

Expertise in automated solid phase peptide synthesis is an advantage, but candidates with related experience in the fields of organic/polymer chemistry or process automation are encouraged to apply. A basic knowledge of molecular biology is preferred but not essential.

An appointment will be made for an initial period of three years.

Send complete applications to **GBF, Personnel Dept., Mascheroder Weg 1, D-3300 Braunschweig, FRG or contact Dr. Ronald Frank** (Tel. FRG 531/6181-220) for further information.

(W7352)A

UNIVERSITY COLLEGE LONDON DEPARTMENT OF ANATOMY & DEVELOPMENTAL BIOLOGY

Postdoctoral position in Developmental Biology

Applications invited for a 3 year position (funded by the MRC) to study "Muscle differentiation and patterning in chick craniofacial development". The position is available now but a later starting date is possible. Salary up to £14,703 pa (plus £1,767 pa London Allowance) depending on qualifications and experience.

Send CV and names of 2 referees to **Dr C. Tickle, Department of Anatomy & Developmental Biology, UCMSM, Windeyer Building, Cleveland St., London W1P 6DB or phone 071-636 8333 X3135** for further details. Equal opportunities employer. (3639)A

KINGSTON
P O L Y T E C H N I C

■ *FACULTY OF SCIENCE*
SCHOOL OF LIFE SCIENCES

Lecturer/Senior Lecturer in Human Physiology - Ref: JS809/N.
and
Lecturer/Senior Lecturer in Biomedical Sciences - Ref: JS808/N.

Up to £21,414 p.a. (inc. London Allowance)

We are looking for two people to join an enthusiastic staff in an expanding department. You should have an honours degree in a relevant subject and a postgraduate qualification or professional experience. You will be expected to contribute mainly to the teaching of three life science degrees (Bioanalytical Science, Biomedical Science and Cell and Molecular Biology). There will also be opportunities to participate in our growing research and consultancy activities.

Both appointments will be from 1st September 1990 and may be permanent or for a two year fixed term depending on experience.

For further details and an application form please contact the Personnel Department, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE. Telephone 081-549 1366 extension 2153, quoting appropriate reference number. Closing date: Monday, 11th June 1990.

We are an Equal Opportunities Employer

(3693)A

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
(University of London)
DEPARTMENT OF MEDICAL PARASITOLOGY MEDICAL ENTOMOLOGIST
RESEARCH FELLOW/LECTURER IN MEDICAL ENTOMOLOGY

As part of an expanded programme of support by the ODA on tropical public health at the School, we are seeking a medical entomologist to join an active group of young researchers in the Vector Biology and Transmission Dynamics Unit.

The project will focus on the development of appropriate vector control strategies in urban environments and in the rice-agroeco-system, and will involve close co-operation with specialists from other disciplines. This will be an exciting opportunity for research into innovative methods of disease control based on a sound knowledge of vector biology. The successful applicant will initiate collaborative projects overseas and for this he/she would have field experience.

Salary will be in the range of £12,225-£18,432 inclusive (under review). The post will be a three year contact in the first instance. Applicants should send a full curriculum vitae and the names of two referees to **Ms Helen Abbott, Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT;** 071 636 8636 ext 203, quoting reference RL1.

Informal enquiries to Dr. Richard Lane, 071 636 8636 Ext 350. Closing date 15 June 1990. (3664)A

NLVF
THE AGRICULTURAL
RESEARCH
COUNCIL OF NORWAY

Norwegian Forest Research Institute
&
Plant Molecular Biology Lab NLVF

invite experienced

RESEARCHER — GROUP LEADER LEVEL

to initiate program in "Isolation and characterization of promoters and structural genes relevant to breeding of Norwegian forest trees". Research may involve isolation and characterization of promoters or structural genes from forest trees or from a model species, preferably Arabidopsis. The intention of the program is to establish basic research with the long term goal to contribute to forest tree breeding. The successful candidate should preferably hold a Ph.D. in plant molecular biology.

The position is initially for 4 years. After this period, a successful researcher will be offered a permanent position. Salary is NOK 236.000 – 276.000 per year. The program holds funding for 2 additional positions (personnel to be hired by the project leader). The forestry gene technology group will be one of three groups of the Plant Molecular biology Lab NLVF, which is housed in well equipped laboratories at Agricultural University of Norway at Ås, 30 km south of Oslo. The project is planned in collaboration with the Hormone Physiology Group headed by prof. G. Sandberg at the faculty of forestry, Swedish Agricultural University in Umeå. In this group, a transformation system for poplar (Populus tremula) has recently been developed.

Applicants should enclose a CV and the names of two referees and be addressed to Plant Molecular Biology Lab NLVF P.O. Box 51 N-1432 Ås-NLH, Norwayas soon as possible.

Enquiries should be addressed to Dr. O.-A. Olsen at the Plant Molecular Biology Lab by letter. (W7365)A

FACULTY OF MEDICINE
UNITED ARAB EMIRATES UNIVERSITY

Applications are invited for appointment at **Assistant, Associate and full Professorial rank in the Department of Biochemistry.**

Candidates must possess a medical degree and/or PhD degree or an equivalent higher professional degree from an accredited university or institution of higher learning, or any higher professional certificate such as Membership or Fellowship in the Royal College of the UK, Ireland, Canada, Australia and New Zealand (MRCP, FRCS, MRC Path, MRC Psych, FFARCS, MRCOG, FRCR), or the certificate of the American Board or the Arab Board for Medical Specializations. The language of instruction is English.

Candidates for these positions must be able to demonstrate leadership experience in the development of teaching, research and, where appropriate, clinical service programmes.

Persons interested in additional information on applying should send a curriculum vitae including list of publications, copies of certificates and the names of three current referees to:

Joel H Lanphear, Ph.D.
Associate Dean
Faculty of Medicine
UAE University
PO Box 17666
Al Ain
United Arab Emirates
Fax No: 971 3 638247

Applications will be closed six weeks after date of publication or September 15, 1990 whichever occurs first. (W7366)A

MRC Clinical Research Centre
Watford Road
Harrow, HA1 3UJ

crc

MOLECULAR BIOLOGY OF PSYCHOTIC ILLNESS

Two scientific posts are available within the Division of Psychiatry at the Clinical Research Centre for work on the molecular genetics of psychiatric disease with particular reference to schizophrenia. Experience in linkage methods (including sibling pair analysis) or in in-situ hybridisation and other approaches to RNA in post-mortem brain tissue would be particularly welcome, but applications will also be considered from candidates with scientific or medical qualifications and some relevant experience. The appointments will be for 3 years and salary will be on the MRC Scales appropriate to the candidate's background and experience.

Enquiries should be directed to **Dr. T.J. Crow, Head of Division of Psychiatry,** telephone number 081-869 3510. Application forms and further details are available from the Personnel Dept. 081-869 3431 quoting ref: 125/1/4306. Closing date for completed applications is 15th June 1990.

The Medical Research Council is an Equal Opportunity Employer. (3650)A

DEPARTMENT OF BIOCHEMISTRY
GENE EXPRESSION GROUP

POSTDOCTORAL SENIOR RESEARCH ASSISTANTS OR GRADUATE RESEARCH ASSISTANTS

A 3 year position, funded by SERC is available to study the expression of minor small nuclear RNA genes which have already been cloned and to examine the processes of minor small nuclear ribonucleoprotein particle (snRNP) assembly. The project also aims to investigate the possible modification of these genes to generate altered snRNP particles which might be used to interfere with the expression of other target genes.

Quote ref: RV/697/NAT

An MRC-funded post is also available for up to 3 years to study the structure, expression and function of paired box containing genes from Xenopus. The paired box is one of a number of conserved sequences that are present in the genes controlling animal development and, like the homeobox, may confer DNA binding. During the project nucleic acid and antibody probes will be made and used to determine the pattern of gene expression at the RNA and protein level. Attempts to understand the function of the genes by disrupting expression will also be made.

Quote ref: RV/698/NAT

While experience of Molecular Biology techniques would be advantageous, it is not essential. Candidates possessing, or about to qualify, with a good Honours degree are encouraged to apply as they may be eligible to register for a higher degree.

Informal enquiries to Dr P. C. Turner on 051-794 4367.

Closing date: 12 June, 1990

DEPARTMENTS OF HAEMATOLOGY AND BIOCHEMISTRY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Initial salary within the range £10,458 - £12,381 p.a. (under review)

To work with Dr R. Carr and Dr S. W. Edwards on a project funded by Birthright, examining phagocyte dysfunction in premature babies. In particular the project will investigate the impaired expression of plasma membrane receptors responsible for opsono-phagocytosis using biochemical, immunological and molecular techniques. The successful candidate will join a well equipped research group investigating phagocyte function and dysfunction in a number of human diseases.

Candidates should have or expect shortly to receive a Ph.D. in Biochemistry, Immunology or a related subject.

Informal enquiries to Dr R. Carr on 051-709 0141, ext. 2810 or Dr S. W. Edwards on 051-794 4363.

Quote ref: RV/701/NAT

Closing date: 14 June, 1990

Applications for all the above posts, by c.v. with the names of three referees, should be received by the Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

University of (3698)A

LIVERPOOL

An Equal Opportunity Employer

Max Planck Institute for Limnology, Plön

BIOCHEMIST

Applications are invited for a senior research associate position to study chemical interactions of freshwater organisms (e.g. allelopathy, morphogenetic substances). The candidate must have a Ph.D. degree and experience in trace analysis and chemical characterization of natural organic compounds (GC-MS, HPLC). He/she is expected to lead a well equipped laboratory and to develop a research programme in co-operation with physiological ecologists. Salary according to German university schedule, depending on qualifications. Applicants should send C.V., publication list, and addresses of referees to **Professor W. Lampert, MPI für Limnologie, Postfach 165, D-2320 Plön, FRG.** (W7364)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF BIOLOGICS

EUROPEAN COLLECTION OF ANIMAL CELL CULTURES

BASIC GRADE MICROBIOLOGIST

Applications are invited for the above post within the European Human Cell Bank section of the Collection. The function of the Collection is to store and characterise cell lines, contract cell culture and to act as an Internationally recognised Patent Depository. The European Human Cell Bank is involved with a specific collection of Human Cell lines derived from patients with genetic disorders and chromosome rearrangements. Duties will include the generation of EBV transformed lymphoblastoid cell lines from peripheral blood samples, cell culture and cryopreservation. The post also involves extensive liaison with Clinical Geneticists throughout the UK. There may also be an opportunity for training in molecular biological techniques being developed within the Collection for cell line characterisation.

A graduate scientist is sought, preferably with cell culture experience but this is not essential as full training will be given.

Salary scale is from £9239-£12158 depending on qualifications and experience. The post is initially funded for three years.

National Health Service terms and conditions will apply.

The post may be discussed informally with Dr B Bolton, telephone (0980) 610391 Ext. 2524/2512.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG, (Telephone (0980) 610391, ext 2240, to whom completed forms should be returned by 7th June 1990, quoting Post No. 0507.

(3655)A

WESSEX REGIONAL GENETICS LABORATORY

GRADE B CYTOGENETICIST (PRINCIPAL)

A vacancy has arisen for a Grade B Cytogeneticist to take charge of one of the cytogenetic sections of a large diagnostic laboratory. While it is the policy of the laboratory to rotate principal cytogeneticists among sections, the immediate need is for an individual to take charge of the leukaemic section and priority will be given to applicants with special expertise and interest in the cytogenetics of malignancies.

Applicants must have the Diploma of The Royal College of Pathologists in Cytogenetics or an equivalent qualification.

Salary on the scale of £17,996 – £23,682. Further information may be obtained from Dr Patricia Jacobs or Dr Nina Gregson. Telephone Salisbury (0722) 336212 Ext. 669.

For an Application Form and Job Description please WRITE to the

Personnel Department,
Odstock Hospital,
Salisbury, Wiltshire
SP2 8BJ.

Closing date: 15th June 1990.

(3658)A

RESEARCH ASSOCIATE

needed to characterize molecular mechanisms in gene regulation. Required Ph.D. in Molecular Biology and ability to perform cloning, protein-DNA interactions. Lambda library construction, protein purification including DNA-affinity chromatography, an[illegible] affinity purification of antisera and immunofluorescence. Salary $18,000.00 per year. Job Order No. 3924910. Contact **Utah Job Service, 1234 South Main Street, P.O. Box 11750, Salt Lake City, Utah 84147.** (NW5348)A

ENDOWED CHAIR

CHAIRMAN, NEW GENETICS DEPARTMENT

St. Jude Children's Research Hospital, a private research institute, is creating a new Department of Genetics. The chairperson will be expected to direct an internationally recognized research program in contemporary genetics and will shape the future of genetics research within the institution through new faculty recruitment and by contributions to its overall scientific leadership. The Department of Genetics will be located in a new 240,000 square foot research building to be completed in early 1991, which will also accommodate ongoing basic science programs in Virology and Molecular Biology, Biochemistry, Immunology, Pharmacology, and Tumor Cell Biology, leaving ample space for further future expansion. The Chairman's salary will be provided through committed endowment funds, together with additional resources commensurate with the needs of a dynamic and ambitious individual. Opportunities exist to interact with strong clinical programs in pediatric Hematology/Oncology and Infectious Disease. Applicants should submit a curriculum vitae and the names and addresses of four references to:

James N. Ihle, Ph.D.
Chairman, Department of Biochemistry
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, Tennessee 38105

St. Jude Children's Research Hospital is an Equal Opportunity Employer (NW5325)A

UNIVERSITY OF EDINBURGH

DIVISION OF BIOLOGICAL SCIENCES

LECTURER IN PLANT MOLECULAR BIOLOGY

Applications are invited for a Lectureship in Plant Molecular Biology within the Division of Biological Sciences. The post will be held in an Institute to be formed from the amalgamation of the present departments of Botany, Microbiology and Molecular Biology. The appointment is full-time and is tenable from 1 October 1990. Salary will be within the range £12086 to £16755.

Applicants should possess a degree in Botany, Plant Science, Biochemistry, Molecular Biology or a related subject and have active research interests in the field of plant molecular biology or plant cell biology.

Informal enquiries can be made to Professor J E Dale, Head of Department of Botany (Telephone 031 667 1081 extension 3303).

Further particulars from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS** with whom applications, including a full curriculum vitae (three copies) and giving the names of three referees, should be lodged by 22 June 1990. PLEASE QUOTE REFERENCE NO. 1522.

(3651)A

UNIVERSITY OF SOUTHAMPTON

TENOVUS RESEARCH LABORATORY

Southampton General Hospital

A post is currently available in the Tenovus Research Laboratory for a

Research Assistant

to help with culture facilities for the bulk production of monoclonal antibodies using hollow-fibre technology. The main objective of this project is to provide clinical grade material to a team of scientists and clinicians working on the production of novel antibody derivatives for the treatment of human lymphoma. Experience in cell culture technique and immunoassays would be desirable but not essential. The position is funded for three years and carries a salary, according to age and experience, in the range up to £13,495 per annum.

For further details concerning the project contact Dr M Glennie at the Tenovus Research Laboratory (0703) 704144.

Applications including full C.V. and the names and addresses of two referees should be sent to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH,** by 14 June 1990.

Please quote reference number: 371/JAD/jm. (3687)A

THE UNIVERSITY OF THE SOUTH PACIFIC

Alafua Campus, Western Samoa

LECTURER II/I/JUNIOR LECTURER IN CROP PRODUCTION

(Post 90/32)

A vacancy exists in the Crop Science Section of the School of Agriculture located on the University's Alafua Campus in Western Samoa for a Lecturer in Crop Production.

The appointment will be for one year (October 1990 – September 1991) and therefore provides an ideal opportunity for the incumbent to gain experience of teaching in a regional university and of crop production in the humid tropics.

The duties of the post are to assist colleagues in teaching courses to degree and diploma students in the production of root crops, vegetables and tropical fruits and to assist with ongoing research into the breeding and agronomy of tropical root crops.

Applicants should possess a good first degree in Agriculture and Horticulture with preferably some previous experience in teaching and of crop production in the tropics.

Salary will be in accordance with qualifications and experience in one of the following scales: Junior Lecturer WS Tala 26,058 – 29,237; Lecturer II WS Tala 30,403 – 34,019; Lecturer I WS Tala 35,221 – 47,866. The University also provides gratuity amounting to 15% of basic salary; appointment allowance; partly furnished accommodation at a rental of 12.5% of salary; and a contribution of 10% of basic salary towards an approved superannuation scheme.

Further information may be obtained from the Secretary (Alafua Campus) (Telephone 21671; Telex 251 USP SX; Fax (685) 22933).

Candidates should send THREE COPIES of their curriculum vitae with full personal particulars, names and addresses of three referees and date of availability. In order to expedite the appointment procedure applicants are advised to ask their referees to send confidential reports direct to the University without waiting to be contacted. Applications should be sent to the **Secretary, School of Agriculture, University of the South Pacific, Alafua Campus, Private Mail Bag, Apia, Western Samoa** to reach him no later than *15 June 1990.* Applicants resident in the UK should also send a copy to **Appointments (37884), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.** (W7363)A

FACULTY OF MEDICINE

UNITED ARAB EMIRATES UNIVERSITY

Applications are invited for appointment at **Assistant, Associate and full Professorial rank in the Department of Pharmacology (Clinical, Toxicology).**

Candidates must possess a medical degree and/or PhD degree or an equivalent higher professional degree from an accredited university or institution of higher learning, or any higher professional certificate such as Membership or Fellowship in the Royal College of the UK, Ireland, Canada, Australia and New Zealand (MRCP, FRCS, MRC Path, MRC Psych, FFARCS, MRCOG, FRCR), or the certificate of the American Board or the Arab Board for Medical Specializations. The language of instruction is English.

Candidates for these positions must be able to demonstrate leadership experience in the development of teaching, research and, where appropriate, clinical service programmes.

Persons interested in additional information on applying should send a curriculum vitae including list of publications, copies of certificates and the names of three current referees to:

Joel H Lanphear, Ph.D.
Associate Dean
Faculty of Medicine
UAE University
PO Box 17666
Al Ain
United Arab Emirates
Fax No: 971 3 638247

Applications will be closed six weeks after date of publication or September 15, 1990 whichever occurs first. (W7367)A

UNIVERSITY OF YORK
DEPARTMENT OF BIOLOGY

Molecular Biologist/Parasitologist

Applications are invited for a postdoctoral research assistant to join a group working on the bovine protozoan parasite *Theileria annulata*. This appointment, which is funded by the Wellcome Trust for a period of three years, will involve the characterisation of proteases and protease genes. Particular attention will be focused on the involvement of these enzymes in the processes of cellular invasion, development and virulence. Candidates skilled in the general areas of molecular biology, protein chemistry and parasitology are encouraged to apply. This is a collaborative project with the Centre for Tropical Veterinary Medicine at the University of Edinburgh.

Starting salary within the range £10,458 to £14,169.

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by Friday 15 June 1990 to the Personnel Office, University of York, Heslington, York YO1 5DD. Further particulars are available — telephone 0904-432051. Please quote advertisement reference number 2/6300.

Informal enquiries to Dr F. R. Hall 0904-432864.

(3689)A

LARGE-SCALE OCEAN BIOGEOCHEMISTRY

The biological oceanography and geochemistry/marine chemistry groups at the Scripps Institution of Oceanography, University of California, San Diego, intend to appoint a professor(s) at any level (tenure or tenure track) with a distinguished record or demonstrated potential in original research and in formal graduate teaching. Assistant-level candidates will be expected to show evidence of their potential through a publication record appropriate for their experience and letters of recommendation; associate and full professor level candidates must show evidence of a strong research record. Salary will be based on UC pay scale. Candidate(s) must hold the Ph.D. degree at the time of appointment.

We intend to enhance the study of large-scale, interdisciplinary problems in the water column, such as the use of chemical (including isotopic) tracers to study biologically mediated processes — specifically primary production and the flux of organic carbon — by innovative methods relatively free of enclosure. The successful candidate(s) will have demonstrated capability to devise and/or choose appropriate methods, concern for analytical accuracy and statistical replication, and interpretation of data based on broad knowledge of oceanographic and biogeochemical processes. Experience in large, multi-investigator programs, and in sea-going, is desirable but not essential.

Please send a letter of application with CV including a description of research interest, list of publications, resume of teaching experience and names of five references to:

Chairman, Biogeochemistry Search
Graduate Department, A-008
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093

Closing date for applications is September 1, 1990

An Equal Opportunity/Affirmative Action Employer

(NW5349)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

Division of Biochemistry

LABORATORY SCIENTIFIC OFFICER Grade 2

This post involves assistance with preparation and maintenance of practical classes in the busy biochemistry teaching laboratories at both Guy's and St Thomas's Campuses and general technical duties. Previous experience of a teaching environment will be useful and the ability to work with undergraduate students is important.

Salary in the range £11,241 – £14,792 pa plus £1,580 LWA. For further information contact Mr Ralph Wilson on 071 955 4530.

Application by full CV and names and addresses of two referees to **Mrs E Musselwhite, Personnel Officer, UMDS, Lambeth Palace Road, London SE1 7EH,** quoting reference number G/BIO/551 by 7 June 1990. (3644)A

UNIVERSITY OF CAMBRIDGE

Sub-Department of Animal Behaviour, Department of Zoology

POST-DOCTORAL RESEARCH ASSOCIATE

The associate will be involved with Professor G Horn and Professor P Bateson in a 3-year project on imprinting in chicks, supported by the AFRC and starting on 1st October 1990. Experience of behavioural techniques is essential; a knowledge of vertebrate brains would be an advantage.

Salary according to age, £10,485 to £15,372.

Apply no later than 15 June 1990 with a cv and the names of two referees to **J Andrews, Department of Zoology, Downing Street, Cambridge CB2 3EJ.** (3676)A

UNIVERSITY OF NEWCASTLE UPON TYNE
Cell Biology/Pharmacology/Physiology

Research Associates (2)

Two posts are available immediately for enthusiastic scientists to join a large interdisciplinary group interested in epithelial cell function.

Gastrin receptors in gastrointestinal cancer cell function. Supported by Yamanouchi Pharmaceuticals, Tokyo, in collaboration with Ferring Research Institute, Southampton, this will involve basic characterization of gastrin receptors in gastrointestinal cancers.

Epithelial/microbial interactions: including identification of antigens involved in microbial binding and endocytosis by enterocytes, or

Epithelial solute transport: transcellular and paracellular routes for permeation, and novel drug pumps, in cultured epithelial cells. This letter post is funded under the LINK Programme in Selective Drug Delivery & Targeting.

The successful applicants are likely to have a PhD or other research experience, and will be appointed to the Grade II (£15,372-£20,469 pa) or Grade IA (£10,458-£16,665 pa) scales (both under review) for up to 4 years. Applications, including full curriculum vitae and names of two referees to **Dr. B.H. Hirst, Department of Physiological Sciences, Medical School, Newcastle upon Tyne NE2 4HH** (Tel: 091-222 6993), by 15 June, 1990.

(3704)A

THE UNIVERSITY OF SHEFFIELD
Department of Obstetrics & Gynaecology
NON-CLINICAL LECTURESHIP

Applications are invited for the above post based in the Clinical Sciences Centre, Northern General Hospital, Sheffield. The main teaching initially will be to 5th year clinical medical students. Current departmental research themes relate to gynaecological oncology, gynaecological laser surgery and nutrition in pregnancy, and fetal growth. It is expected that the appointee will have, or be willing to develop, special expertise with a molecular biology or molecular genetics bent. Salary within Non-Clinical Grade A (£10,458 – £15,372 pa) or Grade B (£16,014 – £20,469 pa). Further particulars from **Director of Personnel rvices, The University, Sheffield, 10 2TN** to whom applications, ncluding a full CV and the names/ddresses of three referees should be ent by **15 June 1990**. Please quote Ref **MAP 288/G.**

An Equal Opportunity Employer

(3690)A

MELBOURNE, AUSTRALIA
SENIOR PRINCIPAL RESEARCH SCIENTIST
POLYMER CHEMIST

(1 POSITION)

$A58,113 - $A63,737 p.a.

RESEARCH SCIENTIST/SENIOR RESEARCH SCIENTIST
POLYMER CHEMIST

(1 POSITION)

$A32,471 - $A47,070 p.a.

RESEARCH FELLOWS
POLYMER CHEMISTS

(2 POSITIONS)

$A32,471 - $A39,724 p.a.

RESEARCH FELLOW
COLLOID/PHYSICAL CHEMIST

(1 POSITION)

$A32,471 - $A39,724

RESEARCH FELLOW
SYNTHETIC CHEMIST

(1 POSITION)

$A32,471 - $A39,724 p.a.

DIVISION OF CHEMICALS AND POLYMERS, CLAYTON, VICTORIA

GENERAL INFORMATION: The Division of Chemicals and Polymers is located in Clayton (opposite Monash University, in the State of Victoria, Australia,) and has a staff of 180 people, of whom over 70 are professional scientists. The Division has excellent scientific, library and administrative facilities, and aims to develop new technologies to support the growth of the Australian chemical, polymer and water/wastewater treatment industries.

THE JOB:
Senior Principal Research Scientist, Senior Research Scientist/Research Scientist and Research Fellows - Polymer and Water Programs.

The Polymer Program is engaged in the design, synthesis, characterization and evaluation of a wide range of specialty polymers. Currently these include, functional polymers for surface coating and adhesive formulations, compatibilizing agents for polymer blends, advanced polymer matrices for carbon-fibre composites, and polymeric biomaterials. Applications are being sought from scientists with experience in polymer synthesis, organic synthesis, or the physical/mechanical characterization of polymers to join research teams working in the above mentioned areas.

The Water Program is seeking an organic or polymer chemist to synthesize and evaluate novel ion exchange resins and polymeric flocculants in our water and wastewater treatment program. The synthetic work will involve polymerization and polymer modification reactions. Evaluation of ion exchangers will include physico-chemical measurements and laboratory-scale simulation of industrial wastewater treatment processes.

Research Fellow - Industrial Chemicals Program: The appointee will participate in on-going research in the Advanced Surfactants project, within the Industrial Chemicals Program. This project aims to develop novel surfactants and their applications for the manufacturing sector. Currently the project performs industrial contract research in the area of surface cleaning and also maintains strategic research in surface wetting as support for such contract research. Research directed at developing functional surfactant films and coatings for biosensor and opto-electronics applications is also pursued.

Research Fellow - Bioactive Chemicals Program: We are seeking a highly motivated chemist for the Pharmaceutical Chemicals Project, within the bioactive Chemicals Program. The appointee will have the opportunity to explore novel and innovative organic chemical synthesis, aimed at developing commercial drugs for the AIDS and Hepatitis B market.

THE PEOPLE: Applicants should possess a PhD degree or equivalent qualification in either polymer, organic, surfactant/colloid or synthetic chemistry, plus a proven record of achievement in independent research. Sound written and oral communications are a pre-requisite, as is the ability to work in a team environment and to supervise activities of technical staff.

TENURE: Term appointment of 3 years for the four Research Fellow positions.

Indefinite appointment for Senior Principal Research Scientist and Senior Research Scientist/Research Scientist position. Australian Government Superannuation Benefits available.

FURTHER INFORMATION: Selection criteria and job description are available by contacting Ms Janine Pickering, phone (6 13) 542 2483.

APPLICATIONS: Stating relevant personal particulars and professional details, including names and addresses of at least two professional referees, and quoting reference no. 90/5 should be addressed to:

The Chief,
CSIRO Division of
Chemicals and Polymers,
Private Bag 10,
Clayton, Vic. 3168,
Australia.

(W7360)A

Applications close July 31, 1990.

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AUSTRALIA

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CSIRO 5/1

Deutsche
Forschungsanstalt
für Luft-
und Raumfahrt e V
DLR

The German Aerospace Research Establishment, DLR, invites senior level candidates to apply for the position of

Scientific Director

of the Institute for Aerospace Medicine (Successor of Prof. Dr. K.-E. Klein)

The Institute is concerned with biomedical/biophysical research in the fields of aerospace and underwater operations. The application-oriented work is conducted in an interdisciplinary approach, involving human physiology, medicine, biology, biophysics and psychology. The Institute has five divisions located at Köln and one division in Hamburg with a staff of about 140 including 65 scientists. It is equipped with a number of large scale test and simulation facilities.

The Institute is expected to provide significant bioscientific contributions to the study of relevant environmental factors, like weightlessness, pressure, radiation and biological rhythms. In addition to basic and applied research, the Institute is involved in operational tasks, i. e. selection, training and medical support of aircrews and astronauts, and in user support for bioscientific research projects in space. They are conducted within national and international projects.

Rheinisch-
Westfälische
Technische
Hochschule

The Rheinisch-Westfälische Technische Hochschule Aachen, RWTH, offers the position of

Professor (C4)

in aerospace medicine

The appoinment will at the same time include leave to hold the DLR position, while involving reduced teaching duties.

Applicants should have demonstrated expertise in the areas of medicine/physiology oriented towards aerospace applications, or in related fields such as environmental or occupational physiology/medicine.

A strong commitment to interdisciplinary cooperation is expected, in particular with the University's Medical Faculty as well as with natural and engineering sciences.

DLR and RWTH Aachen are seeking in a joint search procedure a personality with relevant scientific qualification and organizational experience.

Applications, in duplicate, to
Chairman of the DLR Executive Board,
Prof. Dr. W. Kröll, Linder Höhe, D-5000 Köln 90
or to
Dean of the Medical Faculty of RWTH Aachen,
Prof. Dr. med. Dr. rer. nat. H. Greiling, Pauwelsstraße 30, D-5100 Aachen

(W7359)A

UNIVERSITY
OF BRISTOL

LECTURESHIP IN INORGANIC CHEMISTRY

This is an established post in the Department of Inorganic Chemistry for appointment as soon as possible. Initial salary according to age; qualifications and experience expected to be in the range £10,458 – £15,372 on the Lecturers' Grade A scale, which rises to a maximum of £20,469 (salaries currently under review).
Further particulars may be obtained from the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH,** to whom applications, including curriculum vitae and names of three academic referees, should be sent by 15th June, 1990, quoting reference CYF11.

(3662)A

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nature

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL

(University of London)

MANAGER/ESS
ADVANCED BIOTECHNOLOGY CENTRE

Applications are invited for the above newly created post tenable for three years from post-doctoral scientists preferably with a background in protein sequencing. The Centre has its own laboratories and provides a central service for technologies such as the synthesis and sequencing of peptides and oligonucleotides for the Medical School, its hospitals, the Charing Cross Sunley Research Centre and the Kennedy Institute for Rheumatology.

The Manager will have opportunity for collaborative and independent research and will be expected to act in an advisory capacity and undertake post-graduate teaching commitments. The person appointed will be considered for Honorary Lectureship.

Salary on scale £14,646-£18,432 per annum inclusive of London Allowance (under review).

Informal enquiries may be made to Professor Roger Mason (tele: 081-846 7047).

Further details from and applications to **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St. Dunstan's Road, London, W6 8RP,** within three weeks of the appearance of this advertisement. (Quote Ref: 90/44.) (3705)A

PUBLIC HEALTH
LABORATORY
SERVICE BOARD

PHLS CENTRE FOR APPLIED
MICROBIOLOGY &
RESEARCH

DIVISION OF
BIOTECHNOLOGY

MOLECULAR GENETICIST

A research scientist is required to join a team actively engaged in the application of recombinant DNA technology to products and processes of biotechnological interest. A vacancy currently exists within a programme concerned with the overexpression of a commercially important Gram-positive gene in bacterial hosts, principally E. coli, and the creation of novel, multifunctional proteins by protein engineering and gene fusion technology.

The successful candidate should hold a degree in a biological discipline — practical experience in a relevant area would be an advantage.

This post is for a fixed term of 2 years; the salary range is currently on the Basic or Senior Scientist scale £9239 – £12158, or £12644 – £13150 depending upon experience. The salary is currently under review as part of regrading to a new scientific scale.

Candidates seeking further information should contact Dr N.P. Minton (Tel: 0980-610391, ext 2562).

NHS terms and conditions apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Tel: 0980-610391, ext 2240, to whom completed forms should be returned by 7 June 1990, quoting Post No. 0909. (3671)A

UNIVERSITY OF
NEWCASTLE UPON TYNE
DEPARTMENT OF BIOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a PDRA to work on the characteristics of glucosinolate accumulation in oilseed rape, funded by AFRC for up to three years. The work will involve identifying patterns and sites of glucosinolate synthesis in relation to the mechanism of accumulation by the developing rapeseed embryo. The appointee will join a group working in conjunction with agronomists in the Department of Agriculture at Newcastle, as part of a multidisciplinary approach to understanding the accumulation of these secondary products. Applicants should preferably have experience of working with membrane transport systems, with additional expertise in plant tissue culture and HPLC an advantage.

Salary will be at an appropriate point on the Grade 1A scale (£10,458-£16,665 p.a.) according to qualifications and experience.

A full curriculum vitae, with the names and addresses of two referees, should be sent, as soon as possible, to **Dr. H. Griffiths, Department of Biology, Ridley Building, The University, Newcastle upon Tyne NE1 7RU,** from whom further information can be obtained. Telephone 091 222 7893. (3706)A

Post-Doctoral position

available in Harvard Medical School affiliated Spinal Cord Injury Laboratory to work on brain extracellular matrix. Experience in protein isolation and characterization required: **Amico Bignami, M.D., Research Service 151, Department of Veterans Affairs, 1400 VFW Parkway, West Roxbury, MA 02132.** *Equal Opportunity Employer.* (NW5350)A

ACTION THEMATIQUE ET INCITATIVE SUR PROGRAMME ET EQUIPE (ATIPE)

Call for offers: molecular basis of genetically-determined human diseases

Deadline for application : June 15, 1990

Aim: The aim of the present program is to promote new groups involved in basic research in the area of genetically-determined human diseases:

- Hereditary diseases with established genetic transmission (e.g. enzymopathies, myopathies, X-linked mental retardation...)
- Diseases in which genetic factors are important determinants of their pathophysiology in the areas of: Immunology, diseases of CNS, psychiatry, vascular diseases, rhumatology, endocrinology...
- Molecular approaches of the genes/gene products, studies of chromosomes, family studies and animal models (e.g. transgenic animals) providing a basis for understanding the mechanisms of disease and/or new therapeutic approaches will be considered. Research projects in the field of cancers should be restricted to cancers for which a familial transmission has been established.

Applications:

- Applicants should be *post doctoral trainees* and/or investigators in biochemistry, genetics, molecular biology willing to specialize in the research areas supported by the present program. Applicants should propose a training-schedule for a period of 1 to 3 years that will be supported by the ATIPE if accepted. At the end of the training program, applicants will be considered for employment in a CNRS Research Laboratory.
- Applications should be written in French or in English and sent in the form of a declaration of intent with a short description of the scientific background and personal scientific project to: Ariel GILLE, CNRS, 15 Quai Anatole France 75700 PARIS. Tel.: (1) 47.53.10.40.
- The chairman of the ATIPE Committee is Dr F. TAMBOURIN, Institut Curie – Section de Biologie, 26 rue d'Ulm 75231 Paris Cedex 05 Tel.: 43.29.01.93 – Fax: 43.26.80.87.

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
Communication du Département des Sciences de la Vie
15, quai Anatole France — 75700 Paris
Tél. (1) 47.53.15.15 – Télex 260 034

(W7361)A

FACULTY OF MEDICINE

UNITED ARAB EMIRATES UNIVERSITY

Applications are invited for appointment at **Assistant, Associate and full Professorial rank in the Department of Biophysics (Medical Biophysics).**

Candidates must possess a medical degree and/or PhD degree or an equivalent higher professional degree from an accredited university or institution of higher learning, or any higher professional certificate such as Membership or Fellowship in the Royal College of the UK, Ireland, Canada, Australia and New Zealand (MRCP, FRCS, MRC Path, MRC Psych, FFARCS, MRCOG, FRCR), or the certificate of the American Board or the Arab Board for Medical Specializations. The language of instruction is English.

Candidates for these positions must be able to demonstrate leadership experience in the development of teaching, research and, where appropriate, clinical service programmes.

Persons interested in additional information on applying should send a curriculum vitae including list of publications, copies of certificates and the names of three current referees to:

Joel H Lanphear, Ph.D.
Associate Dean
Faculty of Medicine
UAE University
PO Box 17666
Al Ain
United Arab Emirates
Fax No: 971 3 638247

Applications will be closed six weeks after date of publication or September 15, 1990 whichever occurs first. (W7368)A

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immunology limited

MOLECULAR IMMUNOLOGIST

Immunology Limited is a new biotechnology company located in Cambridge, U.K. The Company has a portfolio of novel human therapeutic products which will be developed in an attractive purpose-designed laboratory on the Cambridge Science Park.

The successful candidate will be expected to have at least three years post-doctoral experience and the motivation to build and lead a team within the Molecular Sciences Group.

The Company offers a challenging and exciting work environment. Salary will depend upon experience and will be competitive with the pharmaceutical industry. Please write enclosing c.v. to Dr Alan Munro, Research Director, Immunology Limited, Cambridge Science Park, Milton Road, Cambridge CV4 4GN.

(3708)A

The University of Queensland

Equal Opportunity in Employment is University Policy

Two Tenurable Lectureships in Zoology

Animal Ecologist (Position 25890)

Applicants should be Zoologists with an appropriate doctoral degree and demonstrated research and teaching experience in Animal Ecology, as well as a solid background in biostatistics. The successful applicant will be expected to develop a second level course in Population Ecology, contribute to other ecology and related teaching, develop an active research programme and attract postgraduate students and external research funding.

Developmental Biologist (Position 25990)

Applicants should be Zoologists with an appropriate doctoral degree and demonstrated research and teaching experience in the Developmental Biology of animals. Preference will be given to applicants using an experimental approach. The successful applicant will be expected to develop senior teaching in Developmental Biology, as well as participate in teaching Cell Biology at First and Second Levels, develop an active research programme and attract postgraduate students and external research funding.

Salary: $32,197–$41,841 (each position).

The Zoology Department has 17 academic staff and 22 support staff. Research interests included terrestrial & marine ecology, genetics & conservation biology, systematics & evolutionary biology, animal physiology and palaeontology. Facilities include electron microscope unit, molecular genetics laboratory, marine field stations on Moreton Bay and at Heron Is. on the Great Barrier Reef and a 16m trawler. Recent University initiatives relevant to Zoology include the Centre for Conservation Biology, an Aquaculture Group, an emerging school of Marine Science, the Centre for Molecular Biology and Biotechnology and the Vision Touch & Hearing Research Centre.

Closing Date: 29 June 1990.

Application forms and further details concerning method of application are available from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London, WC1H OPF.

(W7351)A

UNIVERSITY OF SOUTHAMPTON

TENOVUS RESEARCH LABORATORY

POSTDOCTORAL SCIENTIST and POSTGRADUATE STUDENTSHIPS in Tumour Immunotherapy

Following major industrial backing from Italfarmaco s.p.a. and Avalon Biosciences Ltd. two Research Studentships and a Postdoctoral position will be available from October 1990 in the Tenovus Research Laboratory. The main drive of this collaboration is the development of novel multi-specific antibodies, bispecific and trispecific, for the treatment of lymphoma patients in Phase I clinical trials.

Candidates for the **Studentships** should hold or expect to hold a first or upper second class degree in Life Sciences, Chemistry or related subject. These positions carry a stipend of £4,200.

Applicants for the **Postdoctoral** position should hold a PhD in a biological science, with preference being given to those with experience in cellular immunology and cell culture techniques. This position is funded for three years and carries a salary, according to age and experience, up to £18,165 per annum.

For further details concerning the projects contact Dr Martin Glennie at the Tenovus Research Laboratory (0703) 704144. Applications including full C.V. and the names and addresses of two referees should be sent to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH,** by 14 June 1990. Please quote reference number: 370/JAD/jm.

Smoking discouraged. (3688)A

ELSEVIER SCIENCE PUBLISHERS LIMITED

EDITOR

TRENDS IN CELL BIOLOGY

Elsevier Trends journals is the Cambridge-based Division of the International Elsevier Science Publishers Group, one of the world's largest scientific technical and medical publishers. At our Cambridge office we are seeking a full-time Editor for our latest monthly review journal **Trends in Cell Biology**.

Joining the well-established Trends family of journals, which includes Trends in Biochemical Sciences and Trends in Genetics, Trends in Cell Biology will publish commissioned reviews, news and feature articles covering selected aspects of molecular and cellular biology. Applicants should have a thorough grounding in molecular and cellular biology, plus some post-doctoral experience in a relevant area of research. Publishing experience, though an advantage, is not required, but a willingness to learn publishing skills is essential.

Our offices are located close to the Centre of Cambridge and offer an informal yet challenging working environment. An attractive salary is offered and benefits include 5 weeks annual holiday, pension scheme and assistance with relocation expenses where appropriate.

To apply, please send your c.v. plus a covering letter to **Dr. David Bousfield, Publisher, Elsevier Trends Journals, 68 Hills Road, Cambridge CB2 1LA, U.K. (telephone 0223 315961).** Closing date for application, Monday 18 June 1990. (3673)A

CELLULAR SIGNALLING MECHANISMS

BIOCHEMISTS/MOLECULAR BIOLOGISTS

Posts are available at the postdoctoral (1A MRC/BHF/Wellcome Trust) graduate assistant (1B MRC/BHF) and research studentship (MRC collaborative awards with Wellcome Pharmaceutical) levels to join well-founded and active teams concerned with signal transduction systems in the Molecular Pharmacology Group. Projects available cover areas of research into the regulation of insulin receptor function by protein kinase C, cloning and expression of cyclic nucleotide phosphodiesterases, receptor molecular biology (Professor Houslay) biochemical regulation of lipid-derived second messengers in cell line model systems (Dr. Wakelam). The Department of Biochemistry at Glasgow University is large, well-founded and was rated '4' in the last UGC exercise. These three year positions are available from 1st October 1990 in groups headed by Professor Miles Houslay (041 330-5903) and Dr. Michael Wakelam (041 339-8855 Extn. 4718) from whom further details of the posts, and living in the European Capital of Culture 1990, can be obtained.

Applications, including C.V. and names of three referees should be directed to either **Professor M. Houslay** or **Dr. M. Wakelam** at **Molecular Pharmacology Group, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.** (3660)A

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF GENETICS

Grade 3 (C) Research Technician

Applications are invited, from men and women, for the above post in the Department of Genetics. The person appointed will assist Dr. J.F.Y. Brookfield in a project concerning the DNA sequencing of controlling regions of Drosophila genes. Main techniques used will include PCR amplication and dideoxy sequencing of DNA, Drosophila maintenance and culture and chromosome mechanics.

Minimum qualifications: ONC Tech. or equivalent, preferably with experience in molecular biology or biochemistry.

Current salary scale £7,711 to £8,645 per annum for a 5 day, 37 hour, working week.

This is a research funded post which is offered for a maximum of 2 years 8 months. Applications, including a full CV and the names of two referees should be made to **Dr. J.F.Y. Brookfield, Department of Genetics, Queens Medical Centre, Nottingham NG7 2UH** by 8 June 1990. (3682)A

EUROPEAN SOUTHERN OBSERVATORY

The European Southern Observatory (ESO) is an intergovernmental organisation, responsible for astronomical research in the southern hemisphere. The eight member states of the ESO are Belgium, Denmark, France, Italy, the Netherlands, the Federal Republic of Germany, Sweden and Switzerland. The Headquarters is situated in Garching near Munich and the Observatory in Chile is currently equipped with 14 optical telescopes having diameters up to 3.6m plus a 15m submillimetre telescope. A Very Large Telescope (VLT) comprising four linked 8m telescopes plus a number of smaller ones dedicated to interferometry is now under development and is expected to be the largest optical telescope in the world on its completion in the late 1990's.

ESO is looking for a (m/f).

INFRARED INSTRUMENTATION SCIENTIST
(ref. EPD205)

to start working as soon as possible in the Infrared Instrumentation Group at the ESO Headquarters.

Assignment:
The successful applicant will be involved mainly in the development of infrared instrumentation for the ESO VLT. He/she will be expected to contribute to the definition of new instruments and to assume a project leadership role during their design and construction in collaboration with scientific institutes and industry within the ESO member states. It will be possible to carry out a limited amount of personal research.

Experience and knowledge:
Doctorate or equivalent experience in science or engineering plus several years of experience in designing/building infrared astronomical instrumentation. The ideal candidate would be an infrared astronomer with a training in physics or engineering who has actively participated in both the development and use of instrumentation for infrared astronomy. Specific expertise in high resolution spectroscopy and/or with infrared array detectors would be an asset. An excellent command of English is essential.

Remuneration:
The remuneration for this post is commensurate with the background, experience and family status of the candidate. The basic monthly salary (tax-free) will be between DM 4,994 and DM 9,202 to which may be added a non-resident allowance of either 9% (single) or 12% (head of family) as well as some other allowances.

Although preference will be given to nationals of the ESO member states, no nationality is a priori excluded.

Applications, indicating the reference number, should be submitted before July 31, 1990 to:

Personnel Administration and General Services,
European Southern Observatory,
Karl-Schwarzschild-Straße. 2,
D—8046 GARCHING b. München,
Federal Republic of Germany,
Tel.: (89) 320.06.216-9 (W7370)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR PPLIED MICROBIOLOGY & RESEARCH

DIVISION OF BIOLOGICS

MOLECULAR VIROLOGY GROUP

position exists in the Molecular irology group for a Virologist to tudy the structure and synthesis f flavivirus proteins. Work in the olecular Virology group is imed at understanding the prinles of molecular recognition tween virus proteins and the efence systems of the host. Prorammes currently running in the roup centre on using a variety of ecombinant DNA and synthetic hemistry techniques, coupled vith a number of biophysical nethodologies to elucidate those structural features of viral proteins which are important for their interaction with the immune system.

This position will be for 18 months in the first instance with a possible extension for a further 2 years. The appointment is currently on the Basic or Senior Scientist scale: £9,239-£12,158, or £12,644-£16,639 depending upon experience. The salary is currently under review as part of regrading to a new scientific scale.

Whitley Council terms and conditions apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Tel: 0980-610391, ext 2240) to whom completed forms should be returned by 14 June 1990, quoting Post No. 0528. (3697)A

C S A

SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

DIRECTOR

Microbiology Reference Unit

The Blood Transfusion Service supply blood and human blood products to the Scottish Health Service, providing research and advice on clinical applications.

We're now looking for an experienced Scientist to head and run our new Microbiology Reference Unit, based at B.T.S., Law Hospital, Lanarkshire. Principal duties will be to provide a service to all Blood Transfusion Centres in Scotland, collaborating with them on all aspects of Microbiology quality assurance and giving authoritative advice on technical topics/policy matters.

Already extensively involved in Microbiology, you'll be a Post Doctorial graduate, but need not necessarily be medically qualified. Previous experience in the provision of virological testing to a Blood Transfusion service would be an advantage. The ability to control and motivate a professional team is essential, as are good communicative skills.

We offer an appropriate Top Grade Scientist Salary which will reflect on experience and qualifications.

Informal discussion on this post may be held with Professor John D. Cash, National Medical and Scientific Director, to whom you would report, on 031-220 4590.

Application forms are available from Scottish National Blood Transfusion Service, Headquarters Unit, Ellen's Glen Road, Edinburgh EH17 7QT. Tel: 031-664 2317 Ext: 258.

Closing Date: 16 June 1990. (3707)A

COMMON SERVICES AGENCY
Working for Health

BIOACTIVE SURFACES

Postdoctoral position

Applications are invited for an industrially funded position initially for up to 2 years at the Department of Botany, University of Cambridge to generate and study the properties of bioactive anti-microbial surfaces. Experience in immobilisation technology or microbial physiology/biochemistry desirable.

Applications, including c.v. and the names of two referees, should be sent without delay to **Dr. K. Johnstone, Botany School, Downing Street, Cambridge CB2 3EA (Tel 0223 333933)**, from whom further details may be obtained. (3702)A

INSTITUTE OF PSYCHIATRY, DEPARTMENT OF NEUROLOGY

Post doctoral research worker required for project on adnormal excitatory neurotransmission in epilepsy (Prof. B.S. Meldrum). Techniques employed include receptor autoradiogrpahy of human temporal lobectomy and animal experimental material, and stereotaxic microinjection in rats. Duration of appointment 2½-3 years (salary scale 1A enhanced). Funding provided by the Wellcome Trust. Applications in the form of CV including the names of two referees should be sent to the **Personnel Office, Institute of Psychiatry, De Crespigny Park, London SE5 8AF,** quoting reference 90/R11. *Closing date 8 June.* (3678)A

oxford polytechnic

DEPARTMENT OF GEOLOGY

LECTURER/SENIOR LECTURER IN APPLIED GEOLOGY

Ref: 177/GLC
1 year post starting 1.9.90
Salary scale: £9,117-£20,511

Applicants should have:

- Expertise in one or more areas of Applied Geology (e.g. mineral exploration, petroleum geology, industrial minerals, environmental geology, remote sensing).
- Experience in the use of computers in applied geology.

Secondments from industry, government institutions etc., will be welcomed.

Interviews will take place late June/early July.

Closing date: 11th June 1990.

Further information and application forms available from the Personnel Department, Oxford Polytechnic, Gipsy Lane, Headington, Oxford OX3 0BP. Telephone: Oxford (0865) 819699 or (819297 24 hour answer phone service).

All full times posts open to job sharing unless specifically excluded. (3680)A

WORKING FOR EQUAL OPPORTUNITIES

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology & Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG

SENIOR LECTURESHIP AND LECTURESHIP IN THE DEPARTMENT OF IMMUNOLOGY

The Department is seeking two active research scientists with interests in molecular and cellular immunology for appointment at the level of Senior Lecturer and Lecturer tenable from October 1990. The Lecturer post is for a five-year period; the Senior Lecturer post is permanent. Applications are particularly welcome from molecular biologists and immunologists with experience in molecular genetics. The appointees will be expected to conduct competitive research in their own areas of interest and to collaborate with other members of the department on projects investigating basic mechanisms of T cell physiology. There are also opportunities to develop collaborations with groups on both campuses of Imperial College.

The successful applicants would be expected to contribute to teaching immunological aspects of the MBBS and intercalated BSc courses.

Prospective applicants are invited to telephone Profesor J. Lamb (USA (415) 424-8180).

Further written particulars should be obtained from the **Personnel Department** at the above address, to whom applications in the form of a full C.V. (6 copies) with the names and addresses of 2 referees should be sent by 2 July 1990. (3675)A

NEUROBIOLOGY RESEARCH ASSOCIATE

Research Associate required immediately with expertise in molluscan tissue culture, including microdissection and culture of both ganglia and single neurons from the molluscan nervous system. Demonstrated expertise in the transplantation of single identified neurons between ganglia essential. Minimum qualifications: PhD in neurobiology and experience with neuronal culture and transplantation. Salary range **$28,000-30,000** p.a.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. This term position is funded from external sources and is not a University support staff position.

Submit a curriculum vitae and the names of three referees by June 25, 1990, to:

Drs. A. G. M. Bulloch and K. Lukowiak
Department of Medical Physiology
3330 Hospital Drive N.W.
Calgary, Alberta T2N 4NI. (NW5343)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY

Postdoctoral Research Fellow

This position will be funded by the Royal Society for a period of three years to work on protein and nucleic acid interactions involved in RNA splicing (see Cell (1987) *51*: 1019–1026; Nature (1989) *342*: 819–821 and Proc. Natl. Acad. Sci. USA (1990) *87*: 2216–2219). Experience in protein-nucleic acid interactions, nucleic acid biochemistry, protein chemistry or fungal genetics would be particularly appropriate.

Applications, including full cv and names, addresses and telephone numbers of two referees, should be sent to **Dr Jean Beggs, Department of Molecular Biology, University of Edinburgh, Darwin Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JR** by 14th June 1990. PLEASE QUOTE REFERENCE NO. 5771. (3557)A

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BIOLOGY

Postdoctoral position (Grade 1A) in comparative immunology/haemopoeisis to study the haemopoeitic system of the cockroach, funded by the Agricultural and Food Research Council. Opportunity to acquire experience in monoclonal antibody technology in vitro cell culture. Period of award — 1 year with a possibility of renewal. Starting date 1st October 1990.

Applications, including C.V., should be sent to **Dr. B. Chain, Department of Biology, Medawar Building, University College London, Gower Street, London WC1E 6BT.** *Equal Opportunities Employer.*

Telephone No. 071 387 7050 ext. 3550. (3640)A

DANBIOSYST UK Ltd, Nottingham

Microsphere Technology and Diagnostic Imaging

DanBioSyst is a small, dynamic company specializing in the development of delivery systems for peptide and protein drugs and vaccines. It is situated on the Science Park adjacent to Nottingham University. For our programme in diagnostic imaging we are looking for a young, enthusiastic scientist with experience in Microsphere Technology and the biological application of surface chemistry. A PhD in Pharmaceutical or Colloid Science or Chemical Engingeering would be an advantage.

Please send full CV to the **Managing Director, DanBioSyst UK Ltd, 6 William Lee Buildings, Highfields Science Park, Nottingham NG7 2RQ.** (3653)A

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

RESEARCH APPOINTMENTS IN THEORETICAL CHEMISTRY

Applications are invited for a 3 year SERC/ICI cooperative funded **Postdoctoral Research Fellow** to work on theoretical studies of new materials of Molecular Electronics. Applicants should have a background in quantum chemistry or related fields. Salary: in range £10,458-£12,381 per annum. (Ref R21/90).

Applications are invited for a **CASE (SERC/ICI)** award for work on the theory of electronic excited states of organic molecules with applications to molecular electronics and spectroscopy (from October 1990).

Both projects will involve participation in a well established collaboration with ICI (Colours and Fine Chemicals), Manchester, where extensive computational and molecular modelling facilities are available.

Further details from **Dr D Pugh, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1XL** (041 552 4400 ext 2290). (3695)A

UNIVERSITY OF GLASGOW

Hugh Fraser Lectureship in Neuroscience

Applications are invited for this newly established post from recent postdoctoral graduates. The Lecturer will be required to undertake research into neuroreceptors, cerebral metabolism and cerebral blood flow in collaboration with other basic scientists and clinicians from the Clinical Neuroscience departments. A knowledge of autoradiographic techniques would be a great advantage.

The post is funded initially for a period of seven years. Starting salary will be within the scale for Academic Lecturer Grade A (£10,458-£12,879).

Further details may be obtained from and applications, including c.v. and the names and addresses of three referees, should be sent to **Professor A. Murray Harper, Wellcome Surgical Institute, Garscube Estate, Glasgow, G61 1QH** within four weeks of the appearance of this advertisement. (3648)A

FACULTY OF MEDICINE
UNITED ARAB EMIRATES UNIVERSITY

Applications are invited for appointment at **Assistant, Associate and full Professorial rank in the Department of Physiology.**

Candidates must possess a medical degree and/or PhD degree or an equivalent higher professional degree from an accredited university or institution of higher learning, or any higher professional certificate such as Membership or Fellowship in the Royal College of the UK, Ireland, Canada, Australia and New Zealand (MRCP, FRCS, MRC Path, MRC Psych, FFARCS, MRCOG, FRCR), or the certificate of the American Board or the Arab Board for Medical Specializations. The language of instruction is English.

Candidates for these positions must be able to demonstrate leadership experience in the development of teaching, research and, where appropriate, clinical service programmes.

Persons interested in additional information on applying should send a curriculum vitae including list of publications, copies of certificates and the names of three current referees to:

Joel H Lanphear, Ph.D.
Associate Dean
Faculty of Medicine
UAE University
PO Box 17666
Al Ain
United Arab Emirates
Fax No: 971 3 638247

Applications will be closed six weeks after date of publication or September 15, 1990 whichever occurs first. (W7369)A

Molecular Biologist, Immunologist or Endocrinologist
Senior Research Scientist
PEPTIDE TECHNOLOGY LIMITED

Peptide Technology Limited is a Sydney based biotechnology company undertaking research, development and production of products for veterinary and human medicine. It has laboratories and production facilities in Sydney and Copenhagen (Carlbiotech A/S). Primary research areas include development of vaccines for manipulation of animal production and fertility, other research is aimed at developing novel therapeutic products for human health care including inflammation, immunoregulation and cancer.

The Company requires an energetic and self-motivated scientist to work in the inter-disciplinary area of molecular biology and either immunology or endocrinology. The successful applicant would be expected to initally concentrate on animal growth and fertility research. In addition to holding a Ph.D. degree in a relevant subject, the successful applicant will have post-doctoral experience in a field relevant to the ongoing research.

The successful applicant will be located at CSIRO's Laboratory for Molecular Biology, North Ryde in association with a research group led by Dr. R. Aston.

An attractive salary will be negotiated depending on qualifications and experience. Other benefits including a generous superannuation scheme, share options and housing assistance is available.

Please write with full c.v. to the **General Manager, Peptide Technology Ltd. P.O. Box 444, Dee Why, NSW 2099, Australia or Fax to + 61 2 9828046.** (W7349)A

Division of Entomology

RESEARCH SCIENTIST SENIOR RESEARCH SCIENTIST

A$32,471–A$47,070

Canberra, Australia

THE DIVISION: The Division of Entomology is the major entomological research organisation in Australia and conducts basic and applied research on the taxonomy, ecology, physiology, behaviour, pathology, genetics and molecular biology of insects and related arthropods.

THE JOB: The appointee will undertake research on diseases and disorders of bees, particularly honey bees. A major component of this research will be to develop an understanding of the physiology and pathology of queen honey bees, particularly in relation to commercial queen bee production. Other aspects of the research will be involved with the development of sensitive detection techniques for bee pathogens and with the isolation and characterisation of bee pathogens.

THE PERSON: Applicants should posses a Ph.D. degree or equivalent qualification with experience in the isolation, classification and molecular characterisation of honey bee pathogens. Knowledge of bee keeping and commercial queen bee production is also essential.

CONDITIONS: Appointment will be for a term of two years, with Australian Government Superannuation benefits available.

FURTHER INFORMATION: Prospective applicants are invited to telephone Dr Ray Akhurst on 61 6 246 4160 for further information about the position. A copy of the detailed job description and selection criteria can be obtained by telephoning 61 6 246 4014. Enquiries can be transmitted by facsimile to 61 6 246 4000.

APPLICATIONS: Should be submitted by the 15th June 1990 and quote reference number P0337. Applications must relate to the selection criteria and provide details of relevant personal particulars, including qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

The Chief
CSIRO Division of Entomology
GPO Box 1700
CANBERRA ACT 2601
AUSTRALIA

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W7355)A

UNIVERSITY OF LEICESTER
School of Biological Sciences
Department of Botany

POSTDOCTORAL RESEARCH ASSOCIATE IN MOLECULAR BIOLOGY

A Postdoctoral Research Associate is required to join a group developing methods for the expression of functional antibody fragments in plants. The ultimate aim is to exploit these methods in controlled manipulation of the plant phenotype for biotechnological and plant protection purposes and as a new tool in the study of plants. Experience in such areas as gene expression, gene fusion and expression-vector development would be particularly appropriate for this post. The post, available from June 1990, is funded for two years by the SERC Biotechnology Directorate. Salary on range RA IA (£10,458-£16,665 p.a.).

Applications (including a C.V. and the names of two referees) or requests for further information should be addressed to **Dr. W. Cockburn, Department of Botany, University of Leicester, University Road, Leicester LE1 7RH.** Telephone enquiries are also welcome (0533-523398). (3703)A

Toxicologist/Chemist/ Product Regulations

Hewlett-Packard, a world leader in thermal ink-jet technology, seeks a highly motivated and proficient individual to assume responsibility for EPA and worldwide registration of new chemicals used in ink-jet products, generate MSDSs, supervise toxicity testing through contractors, and set company policies related to use of chemicals in consumer products. Candidate should have 3-5 years' industrial experience in chemical toxicology, familiarity with workings within regulatory agencies, willingness to learn worldwide chemical regulations, flexibility to work on other projects, and possess a Ph.D. or Master's in toxicology with considerable background in chemistry.

Hewlett-Packard, located in the Pacific Northwest, offers an excellent employment and living environment reputed throughout the industry. Please send your resume to: **Janet Flaherty, Personnel, Hewlett-Packard Company, 1000 N.E. Circle Blvd., Corvallis, OR 97330.** Hewlett-Packard Company is an Equal Opportunity/Affirmative Action Employer.

There is a better way.

HEWLETT PACKARD

(NW5339)A

THE UNIVERSITY OF SHEFFIELD
Department of Molecular Biology and Biotechnology
RESEARCH ASSISTANT — GRADE IB

Applications are invited from suitably qualified graduates (or equivalent) to work on a project relating to the use of radiolabelled antibodies in the imaging and treatment of leukaemia. The project aims to examine the molecular basis of the non-specific uptake of these reagents by the mononuclear phagocyte system which can result in poor tumour resolution. Practical experience in biochemistry or immunology would be an advantage. Funding is available from the Leukaemia Research Fund for 1 year in the first instance.

Salary on scale £9,816-£12,381 pa (under review).

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to **Dr L J Partridge, Department of Molecular Biology and Biotechnology, The University, Western Bank, Sheffield S10 2TN.** Informal enquiries can be made to Dr L Partridge (0742-768555 ext. 4185). Reference R1019/G.

An Equal Opportunity Employer. (3667)A

Universitäts-Kinderklinik Zürich, Abteilung Immunologie
POST-DOCTORAL RESEARCH POSITION IN MOLECULAR BIOLOGY/CELLULAR IMMUNOLOGY

Applications are invited from candidates with a recent PhD, a strong experience in molecular biology, interests in cellular immunology/ cell biology and computer skills. The position is part of a research project on the molecular biology of inborn errors of phagocyte (macrophage) functions and is financed by the Swiss National Science Foundation.

The appointment will be for two years initially, starting on June 1st 1990 or by agreement; salary conforms to the SNF norm.

Please send your application including a CV, a list of publications and the names of two referees to:

Prof. R. Seger, Abt. Immunologie, Kinderspital, Steinwiesstr. 75, 8032 Zürich, Schweiz (Tel. 01/259 71 11). (W7362)A

MARINE BIOLOGIST
with background in marine birds and/or mammals
MOSS LANDING MARINE LABORATORIES (MLML)

is seeking a tenure track, probationary appointment at the **Assistant** or **Associate Professor** level to provide quality undergraduate and graduate instruction, and to pursue a vigorous research program on some aspect of sea birds and/or marine mammals. Teaching responsibilities include courses in marine birds and mammals and statistics. A Ph.D. is required. The salary range for Assistant Professor level is $30,276-$41,844; Associate Professor level is $38,112-$52,896/ academic year. The position may be filled as early as Spring 1991.

Moss Landing Marine Laboratories is located on Monterey Bay, an area noted for abundant, diverse populations of sea birds and marine mammals. The Laboratories are operated by a consortium of California State University campuses including Fresno, Hayward, Sacramento, San Francisco, San Jose, and Stanislaus.

Send CV, representative reprints and the names of three referees by September 1, 1990 to **Dr. John H. Martin, Director, MLML, P.O. Box 450, Moss Landing, CA 95039.**

The University is especially interested in hiring faculty members who are aware and sensitive to the educational goals and requirements of an ethnically and culturally diverse population. An Equal Opportunity/ Affirmative Action/Title IX employer.

(NW5344)A

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE
(University of London)
LECTURESHIP IN PHYSICAL CHEMISTRY

Applicants will be expected to have a doctorate and some postdoctoral experience. Salary will be within Lecturer A scale (£12,245 – £17,139) or Lecturer B scale (£17,781 – £22,236) inclusive of London Allowance, (under review).

Applications (including curriculum vitae, list of publications and names of two referees and requests for further details to **Professor D Phillips, Department of Chemistry, Imperial College, London SW7 2AY.**

Telephone 01-225-8336.

Closing date: 7 June 1990. (3694)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF BIOLOGICS
MOLECULAR VIROLOGY GROUP

A position exists in the Molecular Virology group for a Virologist to study the structure and synthesis of flavivirus proteins. Work in the Molecular Virology group is aimed at understanding the principles of molecular recognition between virus proteins and the defence systems of the host. Programmes currently running in the group centre on using a variety of recombinant DNA and synthetic chemistry techniques, coupled with a number of biophysical methodologies to elucidate those structural features of viral proteins which are important for their interaction with the immune system.

This position will for 18 months in the first instance with a possible extension for a further 2 years. The appointment will be made either to the Basic or Senior Scientist scales £9,239 - £12,158 or £12,644 - £16,639 depending upon experience. The salary is currently under review as part of regrading to a new scientific scale. Candidates will be expected to have an honours degree in a suitable subject.

Whitley Council terms and conditions apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Tel: 0980-610391, ext 2240, to whom completed forms should be returned by 7 June 1990, quoting Post No. 0528.

VIROLOGIST AT NORTHWESTERN UNIVERSITY MEDICAL SCHOOL

A tenure track University faculty position in the Department of Pathology offering the opportunity for a major commitment to research with a modicum of hospital service. The successful candidate can be either an M.D. or Ph.D. who has an active research program and experience in the laboratory diagnosis of AIDS. Faculty rank and salary will be commensurate with the level of qualification and academic development. In addition, the candidate will have administrative leadership of a BL-3 level research/diagnostic laboratory. The institution has made a major commitment to AIDS, has an NIH Comprehensive AIDS Center, and an active multidisciplinary group of AIDS investigators. Interested candidates should submit a curriculum vitae by June 30, 1990 to: **Dr. Dante G. Scarpelli, Chairman, Department of Pathology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611.**

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Hiring is contingent upon eligibility to work in the United States. (NW5340)A

PROPOSALS

PROPOSALS FOR DESERT LOCUST RESEARCH

The Scientific Advisory Committee is a joint UNDP/FAO body which supports a donor Consultative Group on Locust Research. UNDP/FAO has limited funds to support research on alternative strategies for Desert Locust Control. The SAC has recommended that these funds be used in the main to support research in forecasting, population modelling, field behaviour and envirnomentally acceptable control. The SAC invites summary proposals covering about 1 page only in these fields. The proposals should describe the project but not consider justification, background or operating mechanisms. They should be sent to, **Secretary SAC, AGP Division, FAO, Via delle Terme di Caracalla, Rome 00100, Italy**.

The SAC would also be willing to consider projects in fields other than those mentioned which it might pass to funding bodies other than UNDP for consideration.

Those submitting summary outline proposals which are considered promising will be invited to submit full proposals in accordance with a format which will be provided by SAC. (NW5347)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

DIVISION OF PATHOLOGY

A vacancy exists in the protein chemistry section for a post graduate or post doctoral researcher to develop technologies for the purification of immunodeficiency virus antigen. The objectives of the project are to produce authentic virus proteins for research purposes and for the development of experimental vaccines. The post is full time, available immediately, initially for two years and funded by the MRC AIDS Directed Programme.

Candidates should have experience in protein purification techniques. Expertise in the area of immunoaffinity purification and FPLC would be desirable but not essential.

Further details and a job description can be obtained from Dr G.H. [illegible]arrar on ext 2560 & 2228.

The appointment will be made to either the Basic or Senior Scientist scales: £9,239-£12,158 or £12,644-£16,639 per annum respectively and will depend on age, qualifications and experience. The salary is currently under review as part of regrading to a new scale.

Whitley Council terms and conditions apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG (Tel: 0980-610391, ext 2240), to whom completed forms should be returned by 7 June 1990, quoting Post No. 0322. (3657)A

FELLOWSHIPS

UNIVERSITY
OF DUBLIN
TRINITY COLLEGE

POSTDOCTORAL FELLOWSHIP

(Department of Chemistry)

Applications are sought for a postdoctoral researcher to work on projects on physical and mechanistic photochemistry funded by the European Community "Science" programme.

The project involves the application of rapid transient spectroscopy (laser flash photolysis and fluoresence lifetime studies) to a range of systems including organic carbonyls, metal complexes, polymers and DNA. The projects involve collaboration with groups in the U.K., Germany and France. Previous experience with photochemical or fast reaction techniques is desirable but not essential.

The fellowship will be tenable until December, 1991, at a salary of IR£15,000 per annum.

Applicants should submit a full *curriculum vitae,* together with the names of three referees to **Professor John M. Kelly, Department of Chemistry, Trinity College, Dublin 2, Ireland,** from whom further details may be obtained. Applications are also invited from recent graduates interested in studying for an M.Sc. or Ph.D. (3665)E

POST DOCTORAL FELLOWSHIP Applied Dielectrics

$A32,471-$A34,887

DIVISION OF APPLIED PHYSICS
NATIONAL MEASUREMENT LABORATORY, LINDFIELD, NSW AUSTRALIA

GENERAL: The Division of Applied Physics applies the methods of physics and measurement science to the solution of problems of importance to Australian Industry and the Australian community. An Electricity Supply Technology Project is supported within the Division's Applied Electricity and Magnetism Program. This project has significant interaction with electricity generation and distribution authorities and with associated manufacturing industry. The Division has a staff of some 300 and is principally located in a modern laboratory in the northern suburbs of Sydney.

The Division's high-voltage laboratory is the largest in Australia and has a 600 kV power frequency test transformer and a 1 MV direct voltage capability. A 1.3 MV impulse generator is shared with the Electricity Commission of NSW.

The Division has initiated a core research project in Applied Dielectrics to focus research on the mechanisms of insulation behaviour in high electric fields. It is envisaged that the study will examine both new and currently used materials.

DUTIES: Participate in applied experimental research related to fundamental aspects of electrical insulation used in high-voltage systems. Develop a theoretical understanding of observed dielectric deterioration mechanisms in general and examine the phenomenon of Electrostatic Charging Tendency in liquids in particular.

QUALIFICATIONS: A PhD degree or equivalent in physics, chemistry or engineering, about to be awarded or awarded within the last two years, with a thesis subject related to electrical insulation preferably at high voltage.

DESIRABLE: Proven ability to plan, perform and analyse experiments on dielectric materials.

TENURE: A fixed-term appointment of 3 years with Australian Government Superannuation benefits available.

FURTHER DETAILS: Dr B. D. Inglis or Dr J. Cook on (02) 413 7211.

APPLICATIONS: Applications, including a full curriculum vitae and the names and addresses of three referees, should be addressed to:

The Chief,
CSIRO Division of
Applied Physics,
PO Box 218, Lindfield,
NSW 2070 AUSTRALIA.

Closing Date for applications: 30 June, 1990.

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W7356)E

AB1 AS90/11

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Research Fellowship (Oxford)

The Imperial Cancer Research Fund is one of the largest research institutions in the UK with wide ranging interests in fundamental, applied and clinical research.

A post doctoral fellowship is available to join a recently established multidisciplinary ICRF group in the new Institute of Molecular Medicine, John Radcliffe Hospital, Oxford. The project is to characterize DNA repair genes from mammalian cells. A bank of novel DNA repair defective hamster cell lines is available to facilitate the study. Existing work in the lab involves the cloning of DNA repair genes, the purification of DNA repair enzymes, the assessment of DNA repair capacity of human tumours, and the analysis of DNA damage inducible genes in human cells. Experience in molecular biology, biochemistry or cell biology particularly of eukaryotic systems, would be desirable.

The fellowship is available for a three year period from 1st June, 1990.

£13,200-£16,800 (3709)E

Informal enquiries can be made to Dr. Ian Hickson on (0865) 752415.

Applications including the names of three referees should be addressed to Dr. I. D. Hickson at the Molecular Oncology Laboratory, Imperial Cancer Research Unit, John Radcliffe Hospital, Oxford OX3 9DU.

SMOKING IS ACTIVELY DISCOURAGED.

Imperial
Cancer
Research
Fund

E•E•R•O

European Environmental Research Organization

Fellowships

The European Environmental Research Organization is a non-profit, non-political organization created by prominent scientists concerned about the environment. EERO's objectives are to promote and encourage international collaboration for the development of high calibre, innovative environmental science in Europe, particularly in the area of chemical pollution. EERO supports research in the following crucial fields: Analytical methods; physical, chemical and biological fate of toxic and polluting compounds; development of processes and technologies for the removal of toxic chemicals and pollutants; and identification of less toxic, readily-degradable alternatives to current industrial chemicals.

EERO awards two classes of exchange fellowships: (i) Long term fellowships for post docs or equivalent, usually one to maximum three years duration. They are intended for advanced training through research and provide a travel grant, subsistence allowance and a modest bench fee. (ii) Short term fellowships of one week to three months duration. These fellowships are to encourage collaborative research between laboratories in different countries within Europe.

Applications for short term fellowships can be made at any time and are decided upon soon after the receipt of the application form. Deadlines for receipt of applications for long term fellowships are August 31, 1990 and February 28, 1991. Successful awards may be activated during the following October and April, respectively.

Application forms and further details may be obtained from **EERO, P.O. Box 191, 6700 HB Wageningen, The Netherlands.**

(W7372)E

Postdoctoral Fellowship Position

is immediately available for a period of up to three year at the Biotechnology Department of Ciba-Geigy Basel, Switzerland to study the molecular biology and the genetics of *Bacillus thuringiensis*. Basic research will essentially concentrate on the expression and regulation of the entomocidal toxin genes.

The suitable candidate will possess a Ph.D. in molecular biology or in related disciplines with a good knowledge of recombinant DNA techniques. This position offers good possibilities to develop own ideas in a stimulating research environment. The establishment of contacts with the academic community as well as the publication of results are strongly encouraged.

Applications, including a curriculum vitae, a description of the current research interests and the names and addresses of three referees should be forwarded under ref. "Nature 4965" to **Mr. H. Schmid, CIBA-GEIGY Ltd., Personnel Department, P.O. Box, CH-4002 Basel.**

(W7350)E

CIBA–GEIGY

University of Edinburgh
Institute of Cellular and Molecular Biology
(Centre for Parasite Biology)
TWO POSTDOCTORAL FELLOWSHIPS

Applications are invited for two MRC-funded Postdoctoral Research Fellowhships to work in a group studying the molecular aspects of DNA replication in the Malarial Parasite. The appointments will be for three years starting on or before October 1st 1990. The starting salary will be up to £12381 p.a. on the AR1A scale. Experience in molecular biology/protein biochemistry/enzymology techniques would be an advantage. Further information can be obtained from **Drs. B. J. Kilbey** (031-667-1081 ext 3691) or **R. Ridley** (031-667-1081 ext 2719), **The Darwin Building, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh EH9 3JR,** to whom applications should be sent.

PLEASE QUOTE REFERENCE NO. 5774 (3652)E

POSTDOCTORAL FELLOWSHIP

TRANSCRIPTION FACTORS IN NORMAL AND LEUKEMIC HUMAN MYELOID CELLS

HARVARD MEDICAL SCHOOL

A postdoctoral position is available immediately for Ph.D.'s in molecular biology to isolate and chara terize transcription factors whic specifically regulate the expression of the monocyte/macrophage marker CD11b (Mol/Mac1), and to investigate the role of these factors in myeloid differentiation and acute myelogenous leukemia. Experience in transcription factor research highly desirable. Send CV and three letters of reference to **Dr. Daniel G. Tenen, Hematology Division, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.**

(NW5346)E

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international
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— EVERY WEEK

FELLOWSHIP AT LA SILLA

A position is available at La Silla for a post-doctoral fellow with an interest in observational astronomy. Experience with IR spectroscopy, or optical photometry will be an advantage. ESO fellowships are granted for a period of one year, normally renewed for a second year and exceptionally renewed for a third and final year.

The successful applicant will be expected to spend not more than 50% of his/her time in support related activities and the rest of the time doing scientific research.

The facilities on La Silla consist of 14 telescopes including the SEST 15-m submillimetre antenna, and the new 3.5m NTT. The computing facilities comprise an HP1000 system with full image processing capabilities (IHAP), a VAX 11/750 and several SUN workstations for image processing (MIDAS).

Close to 20 astronomers, including staff members, fellows and students, work at La Silla. The research projects currently pursued by the astronomical staff at La Silla include low mass star formation (Herbig-Haro objects, molecular outflows, jets), OH/IR stars, symbiotic stars and proto-planetary nebulae, coronal activity in late type stars, chemistry of molecular clouds, formation of massive stars and starburst activity, dynamics of elliptical galaxies, active nuclei, QSOs and gravitational lensing, and observational cosmology.

Applicants normally should have a doctorate awarded in recent years. The monthly basic salary will be not less than DM 4341 to which are added 7% for pension purposes and a non resident allowance of 30–45% as well as a mountain allowance of 5–10%. Applications should be submitted to ESO not later than July 31, 1990. Applicants will be notified by September 1990. The ESO Fellowship Application Form should be used and be accompanied by a list of publications. In addition, three letters of recommendation should be obtained from persons familiar with the scientific work of the applicant. These letters should reach ESO not later than July 31, 1990.

Enquiries, requests for application forms and applications should be addressed to:

European Southern Observatory
Fellowship Programme
Karl-Schwarzschild-Straße 2
D – 8046 GARCHING b. München
Federal Republic of Germany

(W7371)E

UNIVERSITY OF YORK

POSTDOCTORAL RESEARCH FELLOW

AFRC Plant Molecular Biology Intiative
DEPARTMENT OF BIOLOGY

Applications are invited for a post-doctoral research fellowship to investigate the origin and molecular organisation of B-chromosomes in *Allium schoenoprasum*.

Applicants should have, or expect to obtain in the near future, a PhD in a biological subject. Experience of cytogenetics or molecular biological techniques would be an advantage.

The appointment will be available for up to 2½ years on Research and Analogous Grade 1A £10,458–£16,665 per annum.

Two copies of applications with full curriculum vitae and naming two referees, should be sent by Friday, 15 June 1990 to the Personnel Office, University of York, Heslington, York YO1 5DD. Further details are available. Please quote reference number 6/6304.

Informal enquiries may be made to Dr S.M. Bougourd, Department of Biology (tel: 0904 342807). (3696)E

THE UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CLINICAL CHEMISTRY

WOLFSON RESEARCH LABORATORIES – RESEARCH FELLOWSHIP

A new Research Fellowship in the Molecular Biology Group of the Department, located in the Wolfson Research Laboratories. The appointment is available immediately for three years to join an MRC funded project to define allergenic epitopes expressed by native and recombinant allergens important in the pathogenesis of hayfever and asthma.

Applicants should have a PhD in Immunology/Biochemistry or related area and at least two years experience in mammalian tissue culture. Preference will be given to those candidates familiar with heterohybridoma and molecular biological techniques, and a proven interest in allergy.

Informal enquiries should be made to Dr. M.R. Walker on 021-472 1311 (ext 4544) at the Wolfson Research Laboratories, QEMC, Edgbaston, Birmingham.

Salary on Research Fellow 1A scale (£10,458-£12,381 – pay award pending).

Application forms (returnable by 10th June 1990) and further particulars available from **The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT.** Telephone 021-414 6483 (24 hours). Quote ref: M12080.

The University is an equal opportunity employer.

(3681)E

CAMBRIDGE UNIVERSITY
Department of Pathology

RHONE-POULENC VASCULAR BIOLOGY GROUP

The following situations are available in the Rhone-Poulenc Vascular Biology Group headed by Dr David Bowyer.

1. Post-doctoral Research Fellowship to work on the relationship between low density lipoprotein and atherogenesis. The stipend is in the range £10,458-£12,381 depending on age and experience.
2. MRC Collaborative Studentship on 'Regulation of hepatic low density lipoprotein receptor activity'.
3. MRC Collaborative Studentship on 'Effect of cytokines on pherotypic modulation and proliferation of arterial smooth muscle cells and the influence of modified lipoprotein and drugs.

Supplementary payments are made by Rhone-Poulenc to these studentships. Applications with a CV and two references should be made to:- **Dr. D.E. Bowyer, Department of Pathology, Tennis Court Road, CAMBRIDGE CB2 1QP.** (3643)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

3 POSTDOCTORAL FELLOWSHIPS IN YEAST MOLECULAR GENETICS

Applications are invited for three 3-year Postdoctoral Fellowships. One person will study nucleotide sequence elements which modulate gene expression in *Saccharomyces cerevisiae* by destabilising specific messenger RNAs. Two further persons will investigate genes involved in yeast-hyphal dimorphism in *Candida albicans* using recombinant DNA techniques. Dimorphism is important in the pathogenesis of this organism which can be life-threatening in immunocompromised patients. One post is available immediately, and the others are available from 1st October 1990.

The successful applicants will join an active collaborative group studying the molecular biology, genetics, biochemistry and physiology of yeasts. Ideally, applicants should have research experience in one of these areas.

Salary placement on the Research Staff IA Scale depending upon qualifications and experience. Informal enquiries to Drs Brown, Gow or Gooday (0224 273183/44). Applications by letter and CV (two copies) to the **Personnel Department, University of Aberdeen, Regent Walk, Aberdeen AB9 2FX** by 22nd June 1990 quoting reference LG/022. (3666)E

ASSISTANTSHIPS/STUDENTSHIPS

UNIVERSITY OF SHEFFIELD
Department of Chemistry
SERC Postdoctoral Research Assistantship
Total Synthesis of Natural Products Using Tandem Pericyclic Reactions

Applications are invited from qualified and highly motivated candidates to develop novel synthetic methodologies based on Tandem Pericyclic Reactions and apply them to the total synthesis of natural products such as zizaene, gibberellic acid, pseudoguaianolides and insect antifeedants.

The project, funded by the SERC, will give the successful candidate the opportunity to join a young and active research group with wide-ranging interests for up to three years, starting 1 September 1990.

Applications with letters of recommendation should be sent as soon as possible to: **Dr. István E. Markó, Department of Chemistry, The University, Sheffield S3 7HF**. Tel: 0742-768555 ext. 4505. Fax: 0742-738673.

For any other information, do not hesitate to contact directly. (3646)P

UNIVERSITY OF GLASGOW
Department of Chemistry

Postdoctoral Research Assistant

Applications are invited for a Postdoctoral Research Assistantship, supported by the British Technology Group, for work in collaboration with Professor D.J. Robins, on the synthesis of novel bioreducible drugs. The new heterocyclic compounds will be assessed for anti-tumour activity. Candidates should have a strong background in synthetic organic chemistry. The appointment will be available for two years, starting on the 1st August, 1990, or as soon after as possible. Initial salary range £10,458-£12,381, depending on age and qualifications (Range 1A under review, Research Staff Scale) with U.S.S. benefits. Applications including a full *curriculum vitae* and the names of two referees should be sent to **Professor D.J. Robins, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ.** (3647)P

MRC COLLABORATIVE Ph.D. STUDENTSHIP
Gene cloning of ligand-gated ion channels involved in nociception and pain.

A Ph.D. studentship is now available under the supervision of Dr. D. Latchman (Medical Molecular Biology Unit, Middlesex Hospital and University College Medical Schools), and Dr. J.N. Wood (Sandoz Institute for Medical Research). The project concerns the isolation and characterisation of ligand-gated ion channel subunits (5-HT3 and capsaicin) that are involved in the activation of sensory neurons, using the polymerase chain reaction and functional expression in Xenopus oocytes. The eventual aim of such studies is to define loci for the development of novel analgesic drugs. The successful candidate will join groups with extensive experience in PCR cloning, oocyte expression systems and sensory neuron biology. Further information about the project is available from Dr. Latchman (071- 636 8333) or Dr. Wood (071-387 4445). Applications should be sent to Dr. J.N. Wood, The Sandoz Institute, 5 Gower Place, London, WC1E 6BN by June 30, 1990.

(3699)F

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY
3 RESEARCH STUDENTSHIPS

Applications are invited for 3 Research Studentships funded by the Medical Research Council leading to a PhD Degree from Candidates who expect to graduate this year with, or already hold, a good honours degree.

Two positions are available working in Molecular Virology with Dr. A.C. Minson and Dr. T.D.K. Brown.

The Third studentship is a collaboration venture with Wellcome Biotech working on a project entitled The molecular characterisation of salmonella virulence genes — applications for vaccine development and drug delivery? This project is supervised by Dr. C.E. Hormaeche at Cambridge and Dr. G. Dougan at Wellcome.

For enquiries to project supervisors, telephone 0223 333690. Applications in writing with CV and two references as soon as possible to **The Superintendent, Department of Pathology, Tennis Court Road, CAMBRIDGE CB2 1QP.** (3641)F

UNIVERSITY OF GLASGOW
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited immediately for a SERC-funded post-doctoral assistantship. The successful candidate will join a well-equipped team working on experimental deformation density studies covering a wide range of organic and transition metal compounds. Applicants must have a Ph.D. and should have a good background in X-ray crystallography and/or computational chemistry.

The post is tenable for up to three years and can be filled immediately. Starting salary will be in the range £10,458-£11,088 p.a. depending on qualifications and experience.

Applications, including curriculum vitae and the names and addresses of two referees, should be sent to **Dr. Kenneth W. Muir, Chemistry Department, University of Glasgow, Glasgow G12 8QQ, Scotland.** Informal enquiries can be made by telephone (041-339-8855, Ext. 5345 or 4427) or FAX (041-330-4888).

(3659)P

THE UNIVERSITY OF LEED
BIOTECHNOLOGY UNIT
Molecular Electronic
Studentship

As part of the SERC materi
commission initiative
molecular electronics t
Unit has been awarded
earmarked studentship
work on the chemistry of t
flavin adenine dinucleoti
molecule of the enzyme al
hol oxidase. The research v
concentrate on modification
FAD to create a molecular el
tronic switch or memory cel

Applicants will require a go
degree (1st or 2i) in Chemis
with a good knowledge of B
chemistry or Electrochemist
although training in the
areas is available. Inforn
application should be made
telephone or in writing to **D**
R Woodward, Biotechnolo
Unit, University of Lee
LEEDS LS2 9JT. Telepho
(0532) 332590. (3670)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology & Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG
DEPARTMENT OF BIOCHEMISTRY & MOLECULAR GENETICS
MOUSE MOLECULAR GENETICS GROUP

An SERC PhD studentship is available for three years from Octobe 1990 to study the mouse X-linked dystrophin gene. A major project i underway in this laboratory to create transgenic mice carrying site directed mutations in the mouse dystrophin gene via homologou recombination in ES cells. The student would carry out their ow project into the function of one of the domains of the dystrophi protein by the introduction to appropriate site-directed mutations vi homologous recombination. The mouse molecular genetics grou carries out research in genome mapping and mouse transgenesi and seeks candidates with a genetics or biochemistry background.

Applications (C.V. and the names of two referees) should b addressed to **Dr. S.D.M. Brown** at the above address (Tel: 071 723-1252 Ext. 5484). Informal enquiries may also be addressed to D M. Maconochie and Dr. A. Greenfield (Tel: 071-723-1252 Ext. 5471).

(3674)F

UNIVERSITY OF LEICESTER
Leicester Biocentre

Research Studentships in Plant Molecular Biology

Two research studentships are available in the Plant Molecula Biology group of the Leicester Biocentre. The projects, for the stud towards the degree of PhD, are in the following areas:

1. Isolation of genes controlling cell division in plants (SERC CAS award with Shell Research Ltd)
2. Insertional mutagenesis to isolate tissue-specific genes fro potato (funded by Dalgety PLC)

Candidates should have an interest in plant developmental biology and have obtained, or expect to obtain, an upper second or firs class honours degree in molecular biology, botany, biochemistry o genetics. For further information, contact **Dr. Keith Lindsey, Leicest Biocentre, University of Leicester, Leicester LE1 7RH** (phone 0533 522263) to whom applications, curriculum vitae and the names o two academic referees, should be sent by June 15. The expecte starting date is October 1 1990. (3672)F

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH
EDINBURGH RESEARCH STATION
RESEARCH STUDENTSHIPS
RE-ADVERTISEMENT

The Research Station has a substantial programme in embryology, developmental biology, physiology, molecular genetics and transgenics and the following research studentships are offered for October 1990.

1. ***Molecular Genetics of Chicken Inhibin***: Cloning, sequencing and expression of the family of chicken inhibin genes. These clones will be used to study the developmental and tissue specific pattern of gene expression in the chick. Contact: Drs Dave Burt and Peter Sharp.
2. ***Embryo Development and Manipulation***: Analysis of early development in mammals. Techniques of oocyte maturation, embryo culture, embryo stem cell isolation and culture and nuclear transfer are available in the laboratory. Contact: Dr Ian Wilmut.
3. ***Role of MHC Class II Molecules*** in the recognition of bovine T cell epitopes in Foot and Mouth Disease virus derived peptides. Contact Dr Liz Glass.
4. ***Investigating Chromosomal Position Effects in Transgenic Animals***: Transgenes plus the associated flanking chromosomal regions will be isolated from lines of transgenic mice. Large DNA segments will be reintroduced into transgenic mice and expression assessed to investigate the effect of the associated flanking regions derived from the original transgenic mouse. Contact: Dr John Clark.
5. ***Mapping prolactin response elements in a milk protein gene***: cis DNA elements will be defined within the milk protein gene beta-lactoglobulin (BLG) that confers responsiveness to the hormone prolactin by using the gene encoding the prolactin receptor. Contact Dr John Clark.
6. ***Microsatellite Loci as Genetic Markers for Mapping the Pig Genome***: As part of a programme to derive a molecular genetic map of the pig genome, microsatellite loci will be isolated and characterised. The polymorphic markers derived in this manner will be mapped by linkage analysis in large White/Meishan crosses established for the mapping project. The efficacy of microsatellite markers for expanding the polymorphic information content of interesting loci will also be explored. Contact: Dr Alan Archibald.
7. ***Stability of Transgene Expression***: Using established lines of transgenic mice we will test the hypothesis that the genetic background of an animal can be a major determinant of transgene expression level. Contact Dr Maggie McClenaghan.
8. ***Glycosylation Patterns of Proteins Produced in Transgenic Animals***: We will characterise the glycosylation patterns of proteins whose expression has been targeted to different tissues in transgenic mice. Contact: Dr John Clark.
9. ***Insulin-like Growth Factor Expression in Selected Lines of Mice***: IGF-1 gene expression will be examined in mice selected for high and low lean tissue growth to determine its contribution to genetic differences in growth rate. Contact Dr Chris Goddard.

The Research Station occupies new laboratories just outside Edinburgh and students will register with the relevant Biology Departments of the University of Edinburgh nearby, with which we have strong links. In the first instance prospective students should contact the named supervisor on 031-440-2726 at **AFRC IAPGR, Edinburgh Research Station, Roslin, Midlothian EH25 9PS**. (3691)F

UNIVERSITY OF ST. ANDREWS
DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY
PH.D. RESEARCH STUDENTSHIPS

Areas of interest include:- biophysics, molecular genetics, virology, plant and fungal genetics and neurochemistry.

Those interested in applying should have, or expect to obtain, a First or Upper Second Class Honours degree in an appropriate biological discipline.

Applications, including a full Curriculum Vitae, plus the names of two academic referees should be addressed to the **Department, c/o Dr. R. Griffiths, Fife KY16 9AL, Scot-**

UNIVERSITY OF EDINBURGH
DEPARTMENT OF AGRICULTURE
MSc/Diploma in Applied Animal Behaviour & Animal Welfare
(sponsored by CEC, SSPCA, St Andrew Animal Fund, FAW and BVA Animal Welfare Foundation)

Two Studentships are available for this course, starting in October 1990:

MAFF Studentship — Qualifications: Degree in Agriculture/Biological/Behavioural Sciences.

BVA Animal Welfare Foundation — Qualification: Degree in Veterinary Science. This Studentship is open to CEC citizens.

Applications in writing, before 30 June 1990, to **Professor D Wood-Gush, Department of Agriculture, University of Edin-**

DIVISION OF CELLULAR AND GENETIC PATHOLOGY
UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY

Applications are invited for PhD Studentships to commence on or after 1st October 1990, in the following fields:–

LIPID OXIDATION IN ATHEROSCLEROSIS — Macrophages will be studied in culture to investigate the mechanisms by which they oxidise lipids. Techniques will include gas chromatography and fluorescence-activated cell sorting.

ONCOGENES IN BREAST CANCER — A 'transgenic tissue' method for inserting oncogenes into mouse mammary gland will be used to test oncogene effects on hormone control of mammary growth and model breast cancer.

PLACENTAL-MATERIAL INTERACTION — The mechanisms controlling early human placental development will be studied by examining the effects of uterine large granular lymphocytes on placental trophoblast cells *in vitro*.

STEM CELLS AND GROWTH FACTORS — The mechanisms whereby extracellular matrix and novel growth factors control stem cell. Self renewal in epithelia will be investigated using novel *in vitro* and *in vivo* methods.

Enquiries should be addressed to **Dr D E Bowyer, Department of Pathology, Tennis Court Road, CAMBRIDGE CB2 1QP**. (3642)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

Department of Biochemistry

PhD STUDENTSHIPS

Applications are invited for two studentships to work on the following projects in two very active research teams at the RFHSM this autumn:

Project 1: Biochemical studies of medically-important proteins (a choice will be offered) will be combined with protein modelling techniques using a state-of-the-art Silicon Graphics workstation. The grant will be supplemented by £500 p.a.

Project 2: The mechanisms of a mammalian sugar transporter will be investigated using novel expression systems, site-directed mutogenesis, and other techniques, as part of the Membranes Initiative of the SERV. Excellent facilities in new laboratories are available.

Applicants should have (or expect to gain) a First or Upper Second in Biochemistry, Chemistry, Physics or other relevant subject and a strong interest in one or more aspects of the above projects. There will be opportunities for part-time demonstrating. Applications, including a c.v. and names of two academic referees, should be sent as soon as possible to **Dr. S.J. Perkins (project 1, 071-794-0500 ext 3172) or Dr. S.A. Baldwin (project 2, ext 4201), Department of Biochemistry, RFHSM, Rowland Hill Street, London NW3 2PF**, from whom further details can be obtained. (3677)F

SEMINARS & SYMPOSIA

SOFTWARE AND DATABASE DEVELOPERS MEETING
July 16, 1990

National Center for Biotechnology Information

The National Center for Biotechnology Information (NCBI) of the National Library of Medicine, National Institutes of Health, will be holding an informational meeting for software and database developers, July 16, 1990, on the NIH campus. Topics will include:

- GenInfo Backbone DNA and Protein Sequence Database
- Data exchange standards for sequences
- NCBI online services
- NCBI software toolbox project.

This meeting will also serve as a forum for developers to respond to NCBI's plans and suggest additional directions and projects. Both commercial and academic developers are welcome. Registration is required and attendance is limited to 160. For registration information contact: **Zelda Arch, NCBI/NLM, Bldg. 38A,** SEMINARS & SYMPOSIA continued

CELL BIOLOGY OF CARTILAGE AND BONE

A joint meeting of the British Society for Cell Biology and the British Connective Tissue Society

Oxford 17th - 20th September, 1990

List of topics and speakers

Extracellular matrix of cartilage and bone: D.R. Eyre (Seattle), B.R. Olsen (Boston), T.E. Hardingham (London), J. Termine (Bethesda), D.J. Prockop (Philadelphia).

Cell-matrix interactions: J. Lian (Worcester, MA), R. Cancedda (Genoa), M. Horton (London), G.A. Rodan (West Point), R. Wuthier (Columbia).

Growth regulatory molecules: A.B. Roberts (Bethesda), M. Gowen (Bath), J. Tyler (Cambridge, UK), S.A. Jimenez (Philadelphia), G.R. Mundy (San Antonio), J. Wozney (Cambridge, MA).

Development: M. Aydelotte (Chicago), M. Solursh (Iowa), N. Teich (London), P.J. Nijweide (Leiden), S. Dedhar (Vancouver).

Pathology: M. Bayliss (London), J. McClure (Manchester), A.P. Kwan (Manchester), G. Murphy (Cambridge, UK), A.R. Poole (Montreal).

Meeting organisers: F.M. Watt, Imperial Cancer Research Fund, London; M.E. Grant, Manchester University.

For further information and application forms contact **Professor M.E. Grant, Department of Biochemistry & Molecular Biology, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, England.**

Sponsors: The meeting has been made possible by a grant from the Oliver Bird Fund of the Nuffield Foundation. Additional sponsors: Arthritis and Rheumatism Council; Imperial Cancer Research Fund, Wellcome Trust, Ciba-Geigy; Lilly Research Centre; Celltech. (3692)M

REACH ATTENDEES OF THE TISSUE CULTURE ASSOCIATION MEETING

HOUSTON, USA

June 10th-14th 1990

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INTERNATIONAL SYMPOSIUM ON HEREDITARY TUMORS

Florence, the Congress Palace, April 22–24 1991

MAIN TOPICS

Functional Analysis of Tumor Supressor Genes
Epigenetics
Genetics of Predisposition and Progression
Negative Control of Cell Growth

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SCIENTIFIC SECRETARY

Dr. Maria Luisa Brandi, Dept. Clinical Physiopathology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy, Tel. 055-4221906 or 4221816, Fax 055-588156 or 641026

POSTERS

A limited number of free communications will be accepted for poster presentation.
Abstracts must be sent to Dr. Maria Luisa Brandi before December 10th, 1990 (postmarked)

(W7358)M

COURSES & CONFERENCES

INTERFACES 90

International Conference on Interfaces in Medicine and Mechanics
September 9–14, 1990
University of Bologna, Italy

Main Topics

- Material Science of Clinical implants
- Mathematical Modelling of Prosthetic Surgery
- Successes and Failures of Materials in Medicine
- Case histories of Applications
- Numerical Analysis of Biological implants and surrounding tissue
- Interactions in Medicine and Mechanics
- Medical Physics

Enquiries to: **K.R. Williams, INTERFACES 90, Dept. of Basic Dental Science, University of Wales, College of Medicine, Heath Park, CARDIFF, WALES, UK, CF4 4XW**
Tel: (0222) 755944 ext. 2610. Fax: (0792) 295517 (3684)C

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF CHEMISTRY

MODERN ASPECTS OF ORGANIC SYNTHESIS

15th-20th July 1990

This intensive short course includes lectures, tutorials and laboratory sessions covering recent developments in synthetic organic chemistry. It is intended for graduate chemists or those of equivalent experience, working in industry.

For further details please contact **Dr. Tom Gilchrist, Department of Chemistry, The University, Liverpool L69 3BX,** tel. 051-794 3498. (3701)C

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SEPTEMBER 2-7, 1990
Chair: A. Welford Castleman, Jr.,
Pennsylvania State University

COMPLEX FLUIDS
SEPTEMBER 9-14, 1990
Co-Chair: Cyrus R. Safinya (Exxon Research & Engineering)
and
Didier Roux (Centre National de la Recherche Scientifique)

A limited number of fellowships will be awarded for full or partial payment of fixed fee and/or travel. Applicants must apply and be accepted for participation in the conference prior to requesting consideration for a special fellowship. Academic and Industrial Scientists, Postdoctoral Researchers, and Senior Graduate Students in critical financial need are encouraged to apply.

Please send a letter of application for a special fellowship and indicate the nature and level of financial support necessary to **Dr. Alexander M. Cruickshank, Director, Gordon Research Conferences, Gordon Research Center, University of Rhode Island, Kingston, RI 02881** as soon as possible for review by the Special Fellowships Committee. (NW5345)C

INSTITUT COCHIN DE GENETIQUE MOLECULAIRE

INSERM CNRS FACULTE DE MEDECINE COCHIN

ICGM PRACTICAL COURSES

EPITOPE MAPPING USING BACTERIAL EXPRESSION VECTORS

PARIS September 10-21 1990

***Organizers*:**
Keith STANLEY (Sydney)
George BANTING (Bristol)
Richard BENAROUS (Paris)

The course will be based on the epitope mapping technology developed at EMBL by Keith Stanley and coll. (EMBO J.(1987) 6, 1951-1957).

***Course objectives*:**

1—**Construction of epitope libraries from participant's cDNA.** This will be fragmented and cloned into pEX627.

2—**Screening of the epitope library** and colony purification. Participants should bring their own polyclonal serum or monoclonal ascites.

3—**Sequencing of positive clones.**

Maximum number of participants: 20 Course fees: 9000 french francs, lunches included but not accommodations.

Registration deadline: **July 1st.** Application forms are available from: **Dr Richard Benarous ICGM 22 rue Mechain 75014 Paris France.** Telephone: 33 (1) 40 46 04 11. Fax: 33 (1) 40 51 77 49. (W7373)C

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- Engineering Plants for Pest and Disease Resistance
- Agronomic Improvement of Livestock
- Food Engineering

Please send abstracts by September 1 to:

Gina Amatruda
CMC/BIOTECH USA
200 Connecticut Ave.
Norwalk, CT 06856–4900 (NW4985)C

The University of Siena and the Italian Council of Scientific Research (CNR)
organize the
IV SUMMER SCHOOL — GEOLOGY AND PETROLOGY OF CRYSTALLINE BASEMENTS
on
"PETROLOGICAL AND STRUCTURAL ANALYSIS OF PLUTONIC COMPLEXES"
Siena, September 23-October 7, 1990

The School is designed to promote methodological advances in the geological and petrological study of crystalline basements. It will consist of theoretical lectures as well as exercises. Field courses will be held in Corsica and Sardinia.

Lecturers: M. Atherton, B. Bonin, W. L. Brown, J. P. Brun, E. Callegari, R. Capdevila, M. Cuney, D. J. De Paolo, J. J. De Yoreo, A. N. Fernandez, R. Funiciello, C. Ghezzo, L. S. Hollister, P. Le Fort, N. Mancktelow, J. B. Orsini, B. Poty, C. A. Ricci, S. Schmid, A. B. Thompson, V. Trommsdorff, P. Ulmer.

The School is open to 30 students. The cost of the school, including food, lodging and part of the travel expenses for foreign ones, will be provided.

Academic degree in Geological Sciences equivalent to M. Sc. level is required.

Application, curriculum vitae and a list of publications must be forwarded, not later than June 15, 1990, to:
Segreteria Summer School di Geologia e Petrologia dei Basamenti Cristallini, c/o Dipartimento Scienze della Terra, Università degli Studi di Siena, Via delle Cerchia, 3, I-53100 Siena, Italy. Telephone 0577-298803. Telefax 0577-298815. (W7353)C

NATURE

120 YEARS AGO

WITHIN the last few days two members of Her Majesty's Government, the Chancellor of the Exchequer and the Premier himself, have declared, in their places in Parliament, that it is the wish and intention of the Ministry to carry into effect, without further delay, the long-talked-of project of erecting a special building to contain the Natural History Collections of the British Museum.

That this announcement will be received with great satisfaction by the large body of persons interested in the various branches of natural history in this country there can be no doubt, although there may be misgivings in some quarters lest the fullest advantage should not be taken of so grand an opportunity of making the very best museum of the kind in the world. These misgivings are in a great measure justified by the present condition of not only our own, but nearly all other European Zoological Museums.

Long and extensive experience has, or ought to have, taught us the best principle of construction as applied to libraries and picture galleries; but the natural history sciences are in their infancy as compared with literature and art, and the best methods by which their treasures can be housed and exhibited have yet to be learned. The way in which this is done in the new Museum will exercise so great an influence upon the progress of these sciences, that it should not be determined upon without full consideration by those who are most conversant with their present state, and with the probable wants of a future generation of students.

"The New Natural History Museum", W. H. Flower.
From *Nature* May 26, 1870.

100 YEARS AGO

ENGINEER and *Engineering* for May 16 print excellent leading articles on the disastrous accident to the West Coast Scotch express at Carlisle, on March 4; their principal reason being that the Board of Trade Report has just been issued, and that it is in many respects a remarkable document. The accident, as our readers will remember, was due to the driver losing control of the train on entering Carlisle Station, where it ran into a Caledonian engine waiting at the other end of the station. The Report issued by the Board of Trade contains all the available evidence, and the Inspector's opinions as to the cause. The question to be settled was, Why or how did the driver lose control of the train? The Inspector held that the driver was in fault, and this in the face of much evidence that did not support his theory. This evidence he got rid of by the simple expedient of rejecting it as untrue. Our contemporaries clearly demonstrate the real cause of the brake failure, and point out that the Board of Trade Inspector, even after the inquiry, did not understand the construction and working of the North-Western automatic vacuum brake, and that, therefore, his opinion is not worth the paper it is written on.
From *Nature* May 22, 1890.

50 YEARS AGO

IN his broadcast address to the nation on May 19, the Prime Minister when announcing the plans to intern certain aliens emphasized that "there will be many men and women in this island who when the ordeal comes on them, as come it will, will feel a comfort, and even a pride, that they are sharing the perils of our lads at the front. . . ."

We have made a few random inquiries and find that certain aliens (research workers, academic staff and students) in those universities which are already included in the areas announced for special treatment have been interned. It seems impossible that any man of science, no matter what his political or other views may be, could, in the extremely grave circumstances and in view of the amazing revelations concerning the activities of 'fifth columnists' in other countries, take exception to the internment of alien scientific workers and students. Many have established themselves as first-rate scientists, and a glance through the correspondence columns of NATURE will reveal that they have contributed much to the advancement of science while resident in Great Britain. Yet there can be no reason for assuming that there are no dangerous elements among such men of science, any more than among other types of alien refugees. One communication in reply to our inquiry, however, states: "I feel that there is little case for interning *bona fide* pure research scientists. The numbers of such intellectual workers would surely be too few to affect matters." To this one might fairly ask, what is the minimum number below which such people would prove ineffective, and are we always absolutely certain of an alien's *bona fides*, scientific or otherwise. . . . It would be foolish for anyone to ignore the imminence of such peril, and criminal not to anticipate it with the utmost rigour. In the present circumstances, men of science in Great Britain, whether British, naturalized or alien, cannot expect to be shown any form of preferential treatment.
"Internment of Alien Scientific Workers".

THE fibrils of which the artificial 'Nylon' fibre is composed exhibit a remarkably high double refraction. Although their diameter is only about 19μ, they show interference colours of the third order. For the calculation of the double refraction their slightly elliptical cross-section must be taken into consideration. The large and the small diameter can easily be measured under the microscope with slightly twisted fibrils. The double refraction of the fibrils examined amounts to 0.060, the lower index n_δ being 1.520 and the higher index n_γ about 1.580. It is surprising how closely these figures agree with those of silk fibroin and natural cellulose fibres.
"Optics of the Artificial Nylon Fibre", A. Frey-Wyssling.
From *Nature* May 25, 1940.

10 YEARS AGO

SIXTY-FIVE million years ago a major crisis hit the flora and fauna of the Earth. In stratigraphic terms this took place at the boundary between the Cretaceous and Tertiary systems. Evidence of the crisis lies in the more or less simultaneous extinction of major groups of animals and plants including such characteristic elements of Mesozoic life as the dinosaurs, the great marine reptiles, the flying reptiles, the ammonites and belemnites, many bivalve groups, and major groups of marine phyto- and zooplankton. It has been estimated that as many as 75% of Cretaceous species may have been eliminated.

Searching for the cause of this event has been a favourite pastime for many scientists and a great number of hypotheses have been put forward. Some observations suggest a catastrophic mechanism whilst others are indicative of more gradual processes. In the las few years the catastrophists have held sway. The main reason f this is the extremely abrupt wholesale extinction of major groups . . . Hsü in this issue of *Nature*, has used published extinction and trace element data to advance an extreme theory that suggests the extinction of the large terrestrial animals was caused by atmospheric heating during a cometary impact. Poisoning by cyanide released by the fallen comet and a catastrophic rise in the calcite compensation depth in the oceans are separately proposed as the main agents responsible for the extinction of the calcareous marine plankton. Several tests are possible for this hypothesis. First, where is the crater? Hsü assumes the meteorite fell in the ocean, and as much of the Mesozoic ocean floor has disappeared by subduction, the crater may have vanished. The instantaneous extinction of the large terrestrial animals offers another test. Where are all the accumulations of skeletons at the boundary?
"The Cretaceous-Tertiary boundary event", Finn Surly.
From *Nature* May 22, 1980.

IN NEXT WEEK'S ISSUE

Questions posed — and sometimes answered — in the 3 May issue include: When is the next Parkfield earthquake to be expected? Is deterioration of the telomeres an aspect of the ageing process? And how does the barn owl on the cover control his rapid head movement?

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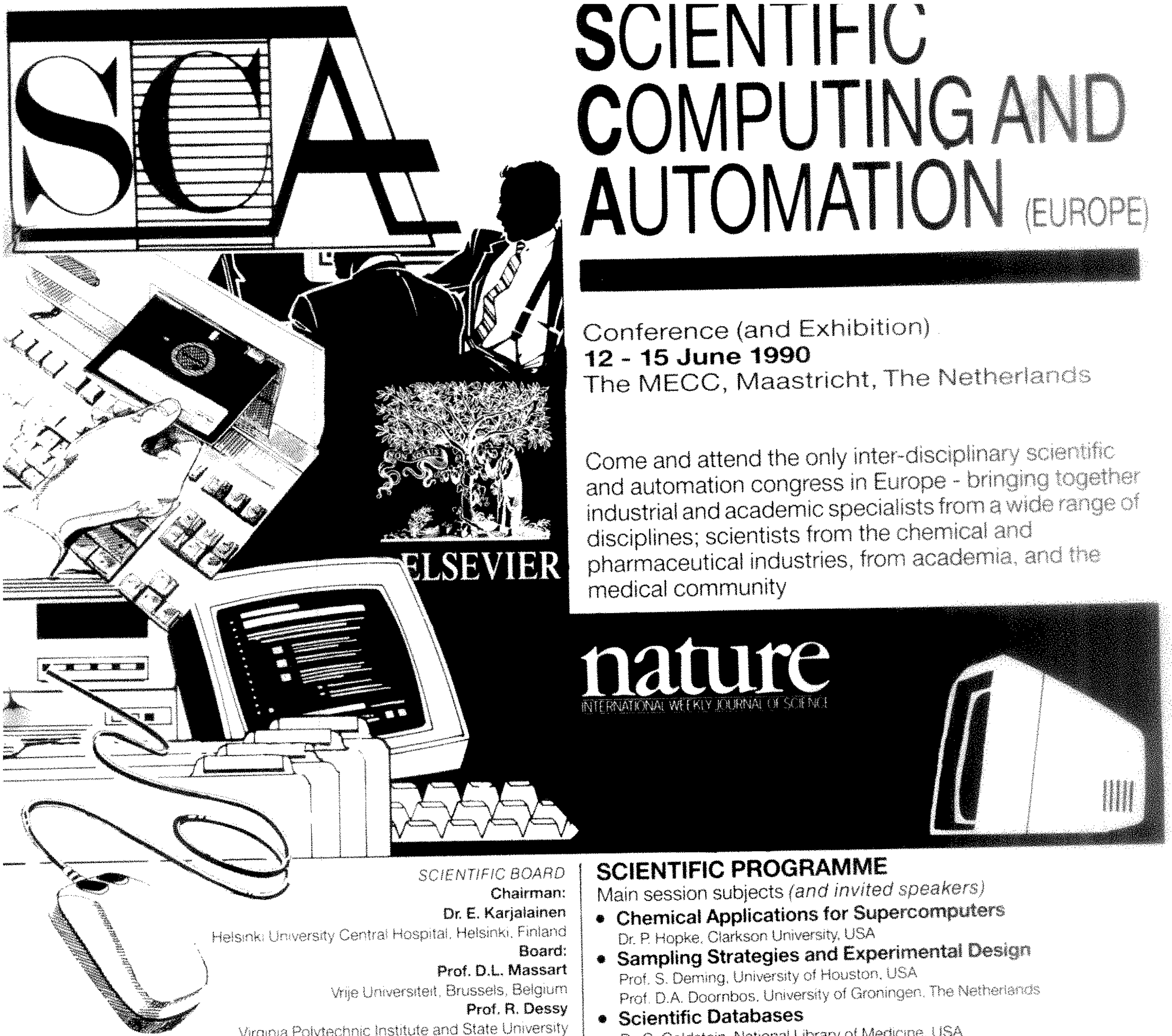
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  Dr. P. Hopke, Clarkson University, USA
- **Sampling Strategies and Experimental Design**
  Prof. S. Deming, University of Houston, USA
  Prof. D.A. Doornbos, University of Groningen, The Netherlands
- **Scientific Databases**
  Dr. C. Goldstein, National Library of Medicine, USA
- **Expert Systems and Statistical Tools for the Interpretation of Laboratory Data**
  Dr. F. Puppe, University of Karlsruhe, W-Germany
  (Diagnostic Problem Solving: Methods and Tools)
- **Robotics and Discrete Automation in the Laboratory**
  Dr. M. Linder, Mettler, Switzerland
  (Laboratory Automation and Robotics - Quo Vadis?)
- **New Emerging Standards**
  Dr. D. Smith, Molecular Design, USA
  (Information Integration:
  Distributed Chemical Information Management System)
- **Chemical Monitoring - Multivariate Sensors and Biosensors**
  Prof. P.R. Coulet, Lyon, France
  From Chemical Sensors to Bioelectronics: A constant search for improved selectivity, sensitivity and miniaturization
- **LIMS and Connectivity Strategies for the Laboratory**
  M. Brooks, Excel International, UK
  Dr. D. Mattes, Smith, Kline and Beecham, USA
- **Workstations for the Scientist**
  Dr. C. Wilkins, University of California, USA
  (Synergistic Use of Multi-spectral Data:
  Missing Pieces of the Workstation Puzzle)
- **Scientific Applications for Neural Networks and Fractals**
  Prof. D.B. Hibbert, University of New South Wales, Australia
  Prof. G. Kateman, University of Nijmegen, The Netherlands
- **Computer Graphics and Image Analysis**
  Prof. J. Encarnacao, Technische Hochschule Darmstadt, W. Germany
- **Europe 1992: Implications for Standards Across Borders**
  J. Noothoven van Goor, AIM Programme, Brussels, Belgium
  (The AIM Programme in Medical Informatics)
- **Validation and Certification**

International Conference on *Bacillus subtilis* Genome.

INSTITUT PASTEUR
25-28 rue du Docteur Roux
Paris - France
September 2-5, 1990

A Conference on the genome of *Bacillus subtilis* organized with the support of the Pasteur Institute and of the Directora General for Science, Research and Development of the Commission of the European Communities will be held at the Paste Institute, Paris, France, September 2-5 1990. There will be three full days of sessions, Monday 3, Tuesday 4 and Wednesda 5, including three poster sessions with time for discussion. The Conference is sponsored by the Ministry for Research ar Technology and the C.N.R.S.

Main topics:

- Regulation of genetic expression, secretion, motility.
- Developmental gene expression, competence, sporulation, germination.
- Genome replication and stability.
- Biotechnological aspects.
- Genome sequencing.

Posters: There will be three sessions which will each be able to accommodate at least 50 posters.

Deadlines: Registration and poster abstract submission: June 30, 1990.

Organizing committe:

C. Anagnostopoulos, R. Dedonder, S.D. Ehrlich, L. Hirschbe G. Rapoport, S. Séror, P. Stragier, J. Szulmajster.

All inquiries should be sent to the Secretariat of th Conference, Unité de Biochimie Microbienne, INSTITU PASTEUR, 25, rue du Dr Roux, 75724 Paris Cedex 15 (Franc Phone: (1) 45 68 88 10, Fax: (1) 45 68 87 90.

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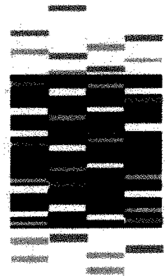

Amersham symposium

Technological aspects of mapping and sequencing genomes

INSTITUT PASTEUR
25-28 rue du Docteur Roux
Paris - France
September 6, 1990

A symposium on technological aspects of sequencing and mapping (nucleic acids) ; organized by Amersham Internatio will be held at the Pasteur Institute, Paris, France on September 6, 1990. It will be a full day session with a dinner organize on the previous evening. (September 5).

Main topics:

- Genetic and physical mapping of genomes.
- New developments in instrumentation for the automation of DNA sequencing.
- Emerging technologies in DNA sequencing.
- Current projects in genome mapping and sequencing.
- Cloning large genomes.

Registration: Please call or send your enquiries to your local Amersham representative.

Registration will be limited to 100 attendees from European countries.

Deadline: June 30, 1990

Invited speakers:

Lectures will be given in English, by international experts sequencing and mapping, including: D. COHEN, Paris (F.); M. ÜHLEN, Stockholm (S S. OLIVER, Manchester (G.-B.); J. SULSTON, Cambridg (G.-B.); A. BANKIER, Cambridge (G.-B.); E. SOUTHER Oxford (G.-B.); A. ROSENTHAL, Cambridge (G.-B H. LEHRACH, London (G.-B.); W. ANSORGE, Heidelbe (FRG); ...

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DUPLICAT

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The next Parkfield earthquake

Ageing cells — shorter telomeres

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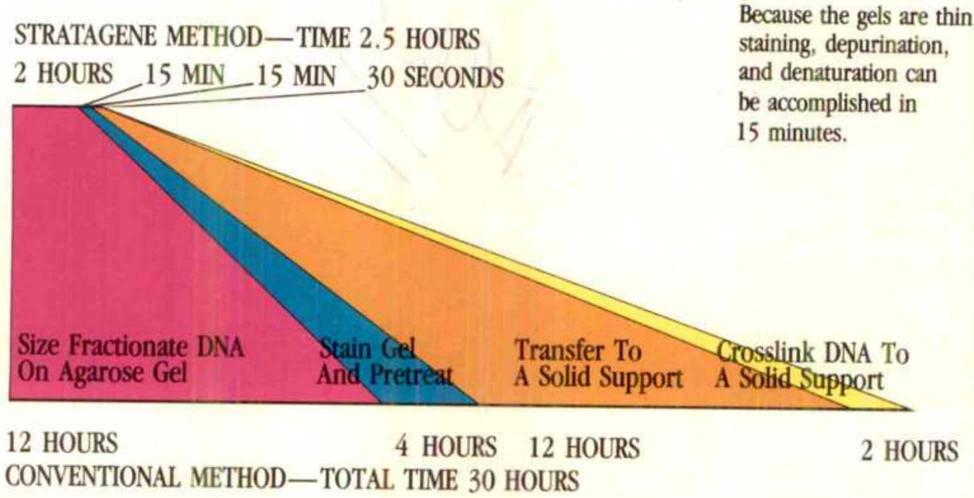

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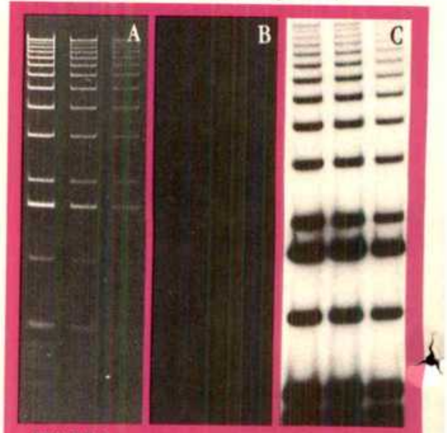
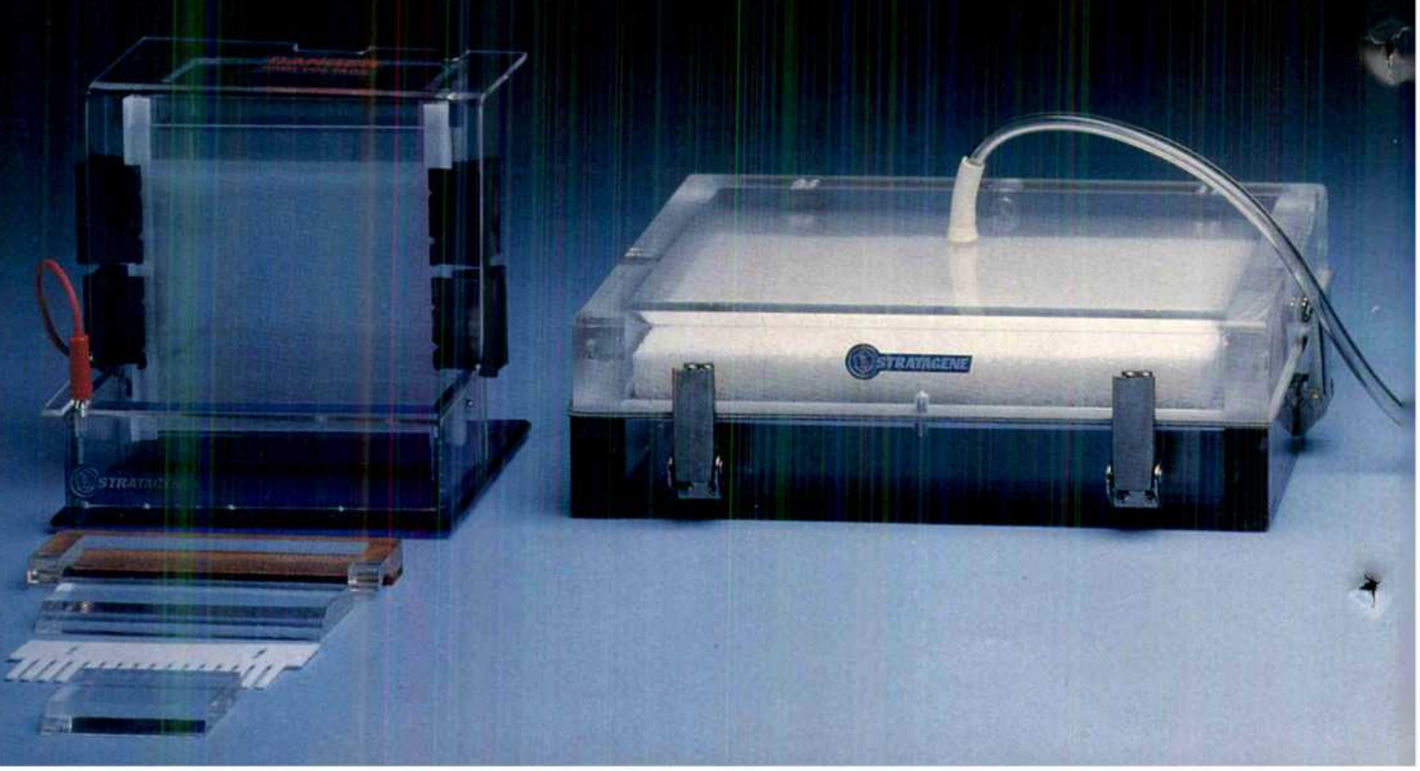


FIGURE 1:

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.
- A. Ethidium stained gel showing high resolution.
- B. Same gel after pressure blotting.
- C. Autoradiogram of membrane after pressure transfer.



PosiBlot™ Pressure Blotter

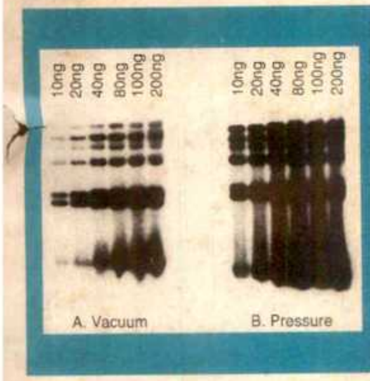


FIGURE 2:
Figure Lengend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

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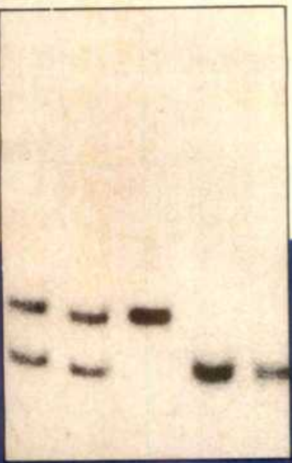

FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

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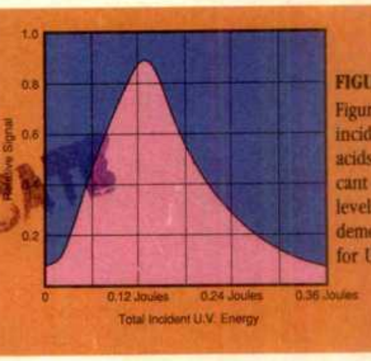

FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

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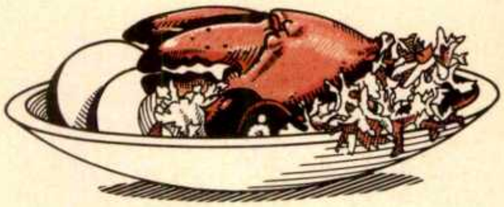

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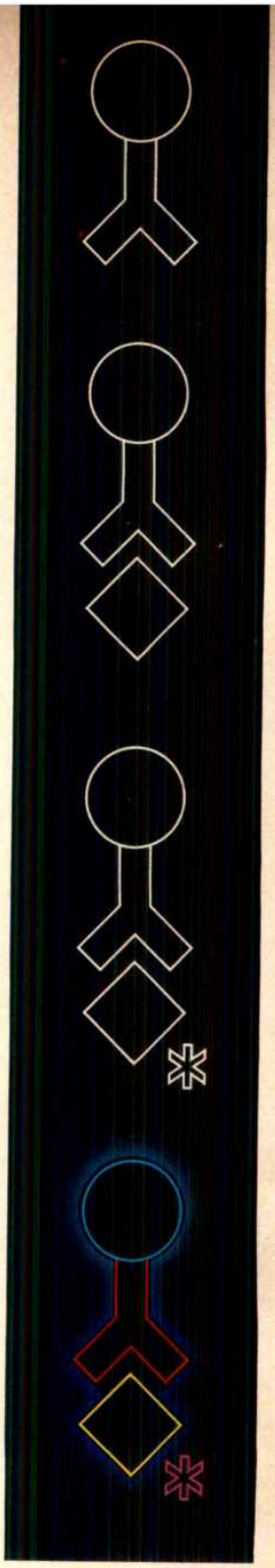

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NEURAL CONTROL OF MOVEMENT
The next Parkfield earthquake
Ageing cells — shorter telomeres

nature

31 May 1990

Vol. 345 Issue no. 6274

◀ Barn owls typically make rapid head movements involving numerous pairs of muscles pulling in different directions and many pulling across multiple joints. Yet at an intermediate level in the brain, the neural code underlying such movements simply represents the movement desired in each of four orthogonal directions: up, down, left and right. See page 434. Photos: E. and P. Knudsen.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

San Andreas prediction

Two strands of evidence — seismic quiescence since 1966 and a reducing crustal deformation rate — are taken to show that the next moderate-magnitude earthquake on the Parkfield segment of the San Andreas fault can be expected within two years. Pages 426, 428 and 383.

DNageing

Telomeres, the specialized end-regions of eukaryotic linear chromosomes, are found to shorten in proportion to the number of cell divisions in ageing cultures of human fibroblasts, suggesting that loss of telomeric DNA may play a part in cell senescence, page 458. On page 456, evidence is presented of a telomere–telomere recombination process, perhaps a telomere replication system or a salvage process.

All is not lost

Little is known about the rate of recovery of biological communities following the deacidification of lakes. However, the study of an acid-stressed lake in a 10-year period during which sulphur inputs were radically reduced suggests that recovery can be rapid and that acid-sensitive species can quickly re-establish themselves. Page 431.

In the garden

A simple 'architectural' mutation in pea plants is used to demonstrate that plant morphology can influence resistance to a herbivore (the pea aphid) by altering the effectiveness of a predator (ladybird beetles). Page 433.

Immunological rescue

A mutant cell with a defect in major histocompatibility complex class I expression that can be corrected by peptide 'feeding' is now known to result from a chromosome 6 abnormality which maps to the MHC itself. Page 449.

Neuronal degeneration

Ciliary neurotrophic factor prevents the degeneration of motor neurons caused by the transection of the facial nerve in newborn rats, suggesting that the high sensitivity of motor neurons to axonal injury in the early postnatal phase is due to shortage of ciliary neurotrophic factor, page 440. A mutant gene that causes late-onset degeneration in a specific group of touch-sensitive neurons in the nematode *C. elegans*, encodes a membrane protein. Its effects can be suppressed by a second mutation, and this phenomenon may throw light on aspects of late-onset human neurodegenerative diseases. Page 410.

Caribbean calibration

A series of coral cores collected off Barbados provides a continuous record of climate and sea level changes for the past 30,000 years. Calibration of carbon-14 dates for these cores with thorium/uranium dates determined by mass spectrometry points to periods when the two scales deviate owing to changes in the Earth's magnetic field, cosmic-ray variations or carbon cycle changes. Pages 405 and 387.

Cool for physicists

Electron cooling focuses and reduces the energy spread of beams of charged particles, opening up intriguing areas of 'new' physics, including studies of extreme states of plasma and experiments with non- and semi-relativistic ions. Page 399.

Osteopetrosis gene

The mutation responsible for the bone-remodelling defect of the *op* mouse, the main mouse model for human congenital osteopetrosis, is in the gene encoding for macrophage-colony stimulating factor. Page 442.

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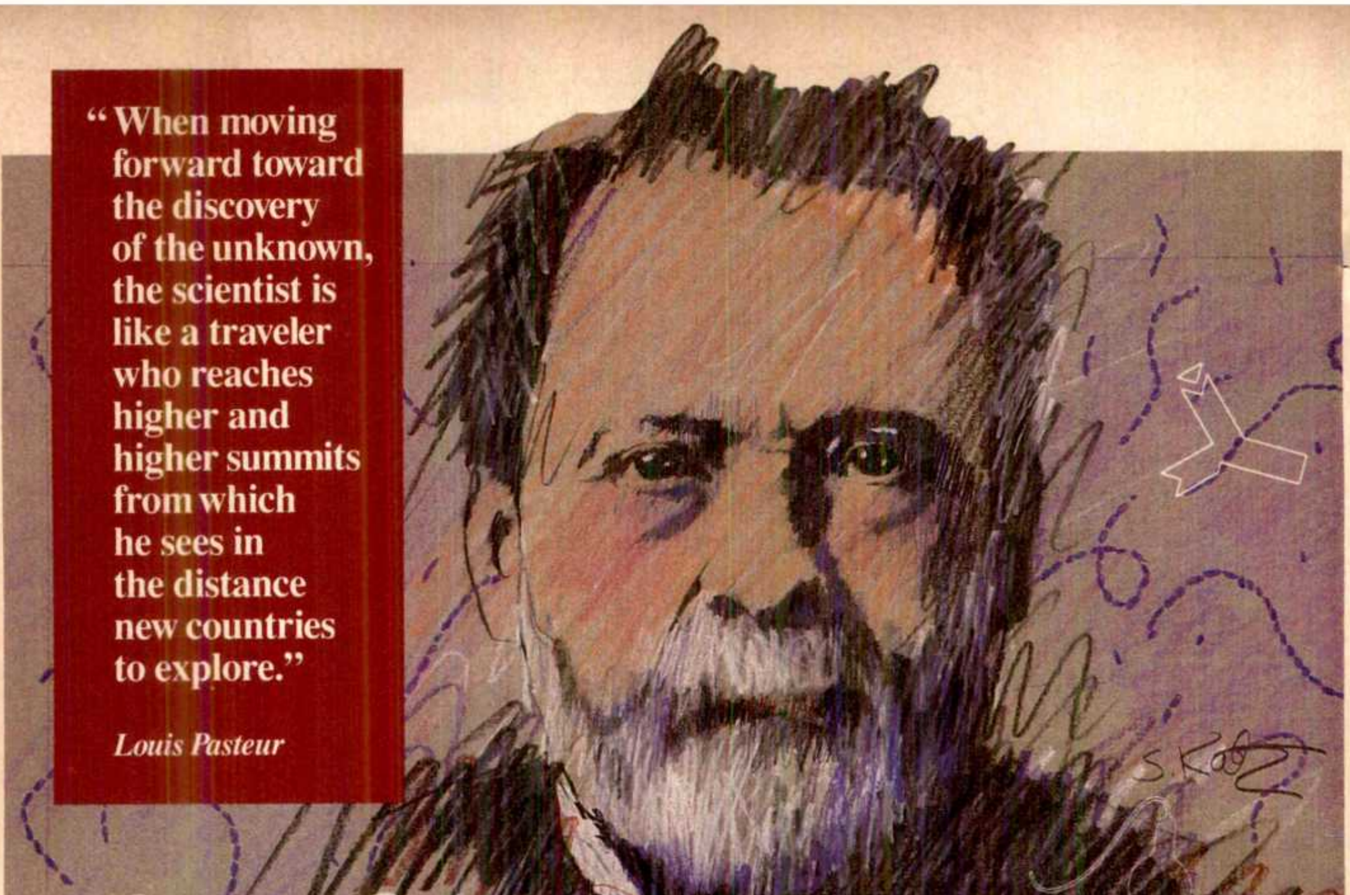

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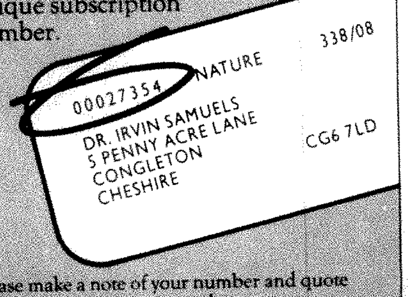

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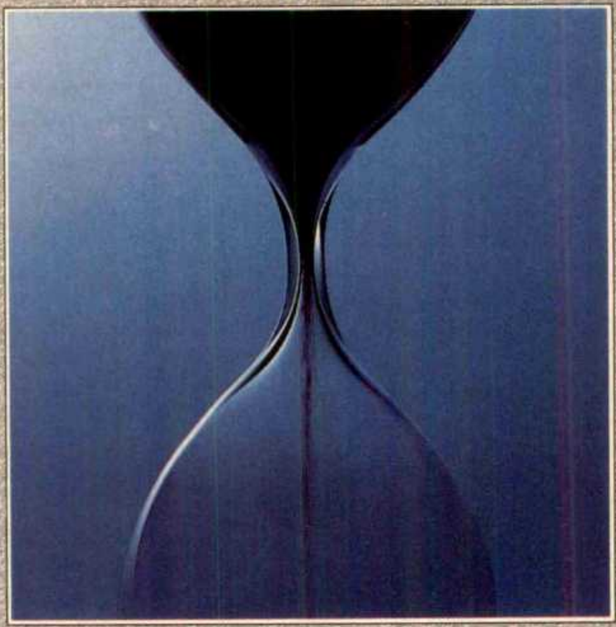

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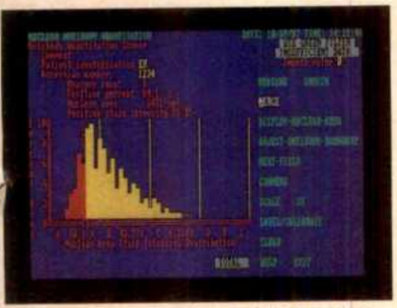

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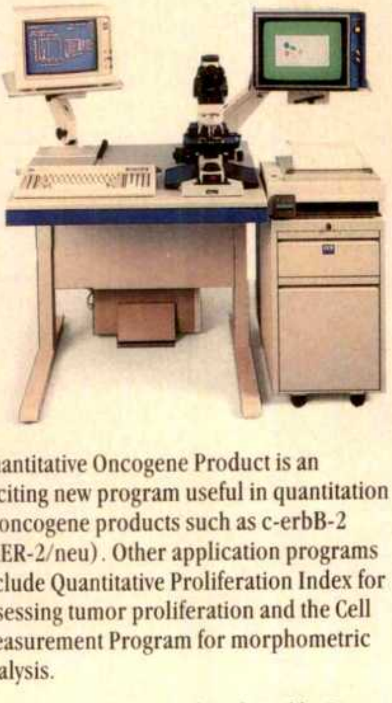

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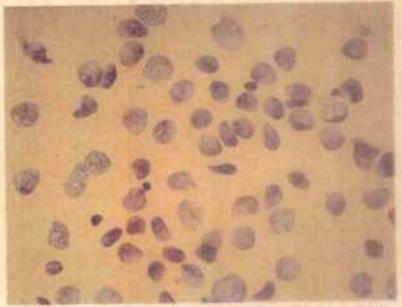
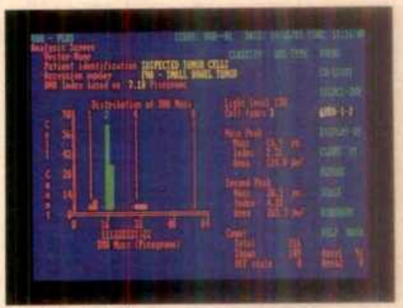


Small bowel tumor (Fine Needle Aspirate) stained for DNA quantitation by the Feulgen technique.



Resulting DNA histogram of the above cells depicting main DNA index (G_0G_1) of 2.21 (aneuploid). Notice low cell proliferation (G_2M).

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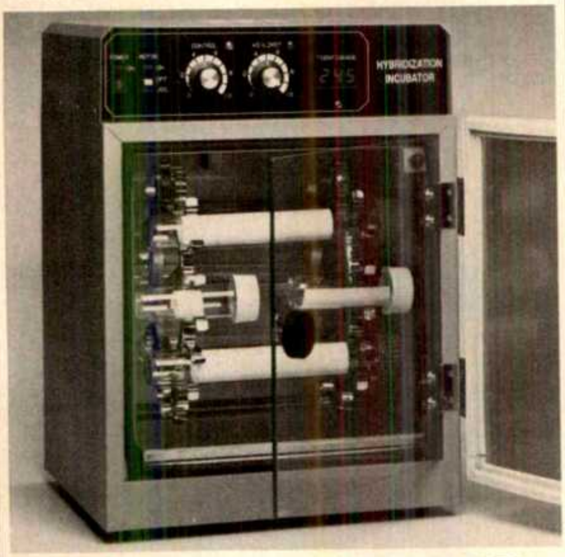

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The great sky greenhouse

Last week's hoop-la notwithstanding, the most urgent need in respect of global warming is to negotiate an international convention on greenhouse gases. *Nature* will play its part with regular summaries of relevant research.

LAST week was a famous week for the greenhouse effect. Mrs Margaret Thatcher, the British prime minister, effectively upstaged Working Group I of the Intergovernmental Panel on Climatic Change (IPCC) of the United Nations by announcing, six hours ahead of time, that Britain is so seized of the reality of the excess greenhouse effect — that proportion of near-surface temperature attributable to anthropogenic rather than natural causes — that it will arrange to restore the emission of greenhouse gases in 2005 to those in 1990 "provided that" this can be done within the framework of an international agreement. Such is Mrs Thatcher's reputation that she was not required to explain how this would be done.

Meanwhile, there are several important issues to keep clearly in mind, not the least of which is that even if the excess greenhouse effect is not yet real, it must at some stage be a factor to contend with. Pangloss, if he were with us still, would surely agree. For the past two years, this journal has been advocating that even the prospect that industrial and agricultural greenhouse gases may change the climate requires that urgent attention should be given to the negotiation of an international convention whose effect would be to limit them. That — an agreed convention with the numbers left blank, to be filled in at a later stage — remains the urgent need. What is being done?

IPCC has three working groups, concerned with the estimation of the extent and nature of likely climatic change (which reported last week — see page 373), with the consequences thereof and the means by which they might be abated. The full IPCC will meet in August. Its report will go to an intergovernmental two-week meeting beginning at the end of October, at which the draft of a convention on greenhouse gases will be drawn to every government's attention. The most important item of that agenda will be 'Date of Next Meeting'. The best hope is that it will not be long delayed, for even a second meeting of the kind proposed cannot be definitive.

Meanwhile, there are several cautions to keep in mind. First, the magnitude of the predicted increase of average global temperature over the next half century is uncertain by a factor of three or thereabouts. (That is why IPCC's Working Group I was seriously in error last week in releasing a general summary of its findings to the general press before telling the professional community how it arrived at its conclusions.) Second, the group concerned with the consequences of climatic change has allowed conditional to be replaced with indicative phrases: for "will be" read "might be". Finally, it is plain that no working politician has more than the sketchiest idea what might be done to ameliorate the effects of an excess greenhouse effect, or to avoid them.

What the years ahead will bring is anybody's guess. Much will depend on the degree to which the computer predictions are borne out by measurements of climate on the surface and of the Earth's radiation balance from Earth satellites. But the most important area of relevant research is also the most neglected. The cost of either adaptation to or avoidance of the excess greenhouse effect may well amount to a substantial fraction of the world's total stock of social capital. Advocates of windmills or of nuclear power as a source of electricity will be much heard of in the years ahead, but the chief burden will fail on the economists who will have to say what are the costs of adaptation or avoidance.

Research, in what Anglo-Saxons call social as well as natural science, will nevertheless play a crucial part in the assessment of what must be an unfolding set of circumstances. This journal is glad to have published in the past few years many of the key documents in this field, but there are many other, perhaps more specialised research reports that naturally find their way into other journals. Beginning next week, *Nature* will therefore publish occasional articles and information designed so as to help readers judge for themselves the quality of the research being carried through in this interesting and important field, its import and the use being made of it by policymakers. Authors of original research, or of research summaries intended for use by committees, official and otherwise, are invited to send copies to the Editor of *Nature*, indicating clearly when their contents may be made public without embarrassment. ☐

German questions

Reunifying the two halves of Germany, could come unstuck, with serious consequences for the rest of us.

THIS week's summit in Washington will make it plain that Mr Mikhail Gorbachev, the President of the Soviet Union, has been compelled by events, by the reservations of his opponents and by the diffidence of his friends to end his compliance with the project to merge the German Democratic Republic (DDR) with the Federal German Republic (FRG). Gorbachev has a point: while arrangements for the security of Central Europe are in the air (and while his own position in the Soviet Union is unclear), the reunification of Germany is premature, to say the least.

Even so, the reunification of Germany is also inevitable. East Germans have for a year been able to move West if they chose. Up to 2,000 a week have been taking advantage of the opportunity. At that rate, the DDR would be emptied of people — except for the very old and, possibly, the very young — in about a decade. The result of that would be a social and military vacuum in Central Europe, especially in the eastern half. West Germany's Chancellor, Helmut Kohl, campaigned strongly during last December's East German elections, but he was merely acting prudently, seeking to avoid an intolerable dilemma.

Yet the pace of events is too fast for comfort. Those who have been engaged in relatively much simpler projects than the merger of whole countries — the merging of, say, departments of physical and inorganic chemistry — know that whole years may go by before a consensus is established. How can it be possible to merge two whole countries in a few months, which is the present intention? Only by taking a whole raft of considerations on trust, by agreeing in advance to agree on certain principles. The trouble with German reunification is that the only tangible agreement so far struck is that up to 6,000 marks of individual personal savings by East Germans will be converted into Deutschmarks at par, with the excess converted at the rate of two for one. It is also agreed that Ostmark salaries will be converted at face value into Deutschmarks, but that is a fiction: either the enterprises at which those concerned work are able to make their way in the competitive world, or they cannot. In the second case, the employees will have to look for work in West Germany, which is the reason why the German question arose in the first place.

The confusion is especially apparent in research. The West German grant-making agency called the Deutscheforschungsgemeinschaft has said that it will, from the beginning of next year, allow East German applications for funds to be considered alongside those from the West. But there is deep disagreement in West Germany about the criteria that should apply. Should the same standards apply to both, or should East German applicants be given an inside track? The first case would spell the end of research in East Germany, the second would breach the principle of excellence on which West German research has prospered in the past decade. Nobody knows how that will come out, nor what the Max-Planck Gesellschaft is up to. What it should be doing, of course, is to accept that the East German Academy of Sciences should be a titularly equal partner until things can be sorted out. In general, that is what the circumstances demand — time to sort things out. ☐

Labour's ambiguity

THE British Labour Party, which hopes to be the next government, should have a policy on research.

THE British Labour Party, temporarily known as Her Majesty's Opposition (but which should constitutionally prefer to be known as Her Majesty's Government's opposition, where the capitalization of nouns is intended), last week produced its provisional agenda for the next British general election. The document is a frank disappointment. On higher education and research, there is only one tangible proposal — the funding councils that at present provide public money to universities and polytechnics will be merged (a sensible idea, on the cards since the late Mr Anthony Crosland invented British polytechnics in 1968). Otherwise, the document says nothing but that there is a need for skill and, therefore, for training as well as for equity in access thereto.

Even calculations of electoral advantage would have commanded a different tack. The present government, in office for 11 years, has ruined the capacity of British research to be imaginative by requiring it (in the proper public interest) to count pennies instead. British academics as different as aristotelians and theoretical physicists have become adept at the allocation of car parking spaces and, by all accounts, exceptionally skilled at telling when the government's apparently perpetual offer of early retirement on favourable terms will allow them to combine a half-time appointment at an American university with growing roses in a clement English county. Demographically, of course, it is even more troubling that younger scholars increasingly regard their first overseas postdoctoral appointment as their chance to play in a different league. The Labour Party's provisional manifesto says nothing on this point, and little to suggest that it would try to make Britain a more interesting place for researchers, in that role, to work. This is all a far cry from the then Mr Harold Wilson's "white-hot technological revolution". There were serious faults in that programme, but the cry was heartening, and electorally appealing.

Last week's document is similarly thin on research as such, which comes through as a handmaiden of industrial policy. The Labour Party (like the present government) would cut back on defence research. But the party's promise, at the last election, that research would be paid for by an industrial levy, is mercifully not repeated. It is nevertheless crucial to those working in research that there should be some undertaking of the scale and the direction in which research funds would be channelled. What the Labour Party should do, between now and the time that electoral considerations will require that it should publish a more substantial successor to last week's document, is to decide what science is for. The party evidently believes, on the evidence of last week's document, that research is either for wealth-creation (the present government's belief) or job-creation. Is that all? ☐

Modest response to climate change threat

- Little new action proposed
- IPCC process questioned

London

An early draft of the Intergovernmental Panel on Climate Change (IPCC) report on responses to global warming which was leaked to the press last week has outraged environmentalists by failing to call for stringent cuts in greenhouse gas emissions. But the outcry may persuade IPCC's working group III to take a tougher line and revise the report at its final meeting in Geneva next week.

On the key issue of carbon dioxide emissions, the draft report does not suggest major changes from current national positions. Western European countries, the report says, may be able to "stabilize or reduce carbon dioxide emissions by early in the next decade". No mention of reductions is made for other regions. Eastern Europe and the Soviet Union may, at best, stabilize emissions over the next two decades, and developing countries may be able to "reduce the annual growth in carbon dioxide emissions from over 3 to around 2 per cent", the draft says.

It is the suggested response from 'North American and Pacific OECD countries' that has attracted most criticism. The report says only that the United States and Japan may be able to slow the growth in carbon dioxide emissions. No timetable is given.

Simon Roberts from Friends of the Earth says "this looks like political intervention with no science at all", a view shared by most environmental pressure groups. Unlike IPCC's other two working groups, which consist mostly of scientists, group III is chaired by a US representative, dominated by civil servants, and has strong Japanese representation.

Group III's suggestions, if carried out, would slow the growth of global carbon dioxide emissions. But the atmospheric concentration of the gas would continue to rise. Climate models produced by IPCC group I, whose report was finalized last week at a meeting in the United Kingdom, predict that emissions of long-lived greenhouse gases (including carbon dioxide) must be cut by more than 60 per cent to stabilize atmospheric concentrations at today's levels.

The group I report predicts the climate change that may result under several different scenarios. If carbon dioxide emissions increase unchecked and atmospheric carbon dioxide concentration doubles by 2025–50, global mean temperature is predicted to increase at about 0.3° C per decade. The most rigid anti-greenhouse strategy examined, reducing carbon dioxide emissions substantially over the next century, gives a predicted rate of increase of about 0.1° C per decade. The implication is that some degree of economic adaptation to climate change, regardless of the action taken to reduce the greenhouse effect, is now probably inevitable.

An early draft of group II's report, looking at the impacts of climate change, was also leaked to the press last week. The draft, to be finalized in Moscow this week, assumes no reduction in carbon dioxide emissions and makes several alarming predictions. Millions of people may be uprooted by flooding, erosion and changes in agriculture, into areas that are "likely to have insufficient health and other support services". Group II's draft says that global agricultural production could be sustained if there are no reductions in greenhouse gas emissions, but many natural ecosystems could not cope with the expected rate of climate change.

Ralph Cicerone, from the University of California, Irvine, one of the scientists who worked on group I's report, believes expectations from "a hasty study like IPCC" may have been too high. He contrasts the IPCC process, which has been running for little over a year, with the long haul towards the three-volume report on chlorofluorocarbons and the ozone layer, which formed the scientific background to the Montreal protocol. The US National Aeronautics and Space Administration began work in 1977, but the final report, compiled with the World Meteorological Organization, was not published until 1986. Cicerone thinks that IPCC must be regarded as a first step towards international agreement, not the final answer. But he hopes that politicians will not use the IPCC reports as "an excuse to do nothing".

A lack of communication between IPCC's working groups may have caused problems. One scientist involved with working group I suggests that the whole process would have been better conducted in serial, rather than parallel with group I feeding its completed report into group II, and so on.

Alan Miller, from the Center for Climate Change at the University of Maryland, thinks that the attitude of the British prime minister, Mrs Margaret Thatcher will now be a key factor (see below). West Germany, the Netherlands and Scandinavian countries are pushing for cuts in emissions, but Britain has been seen to support the United States. IPCC will present a single report to the World Climate Conference in the Autumn, so there will further scope for amendment of group III's position on greenhouse emission controls.

Peter Aldhous

CARBON DIOXIDE EMISSIONS

British pledge on reductions

Bracknell, Berkshire

Prime minister Mrs Margaret Thatcher last Friday (25 May) pledged Britain to return its carbon dioxide emissions back to 1990 levels by 2005, provided the target was part of "a wide international effort". This involved undercutting present UK emission projections by "up to 30 per cent", she said.

Mrs Thatcher was in Bracknell to open the Meteorological Office's new Hadley Centre for climate prediction and research. Dressed in a dark suit matching her sombre message, she spoke of the flooding and drought that could result if global warming is not checked. "People will be crying out, not for oil wells, but for water", she warned.

But the speech gave little detail of how British emissions would be stabilized. An overhaul of transport and energy policy is thought essential by many, but Mrs Thatcher chose instead to highlight the role of the private sector. Worldwide application of a new method of ammonia production, developed in Britain, would have the same effect on carbon dioxide emissions as removing five million cars from the road, she said. Details of government policy on global warming will be given in a white paper (policy document) on the environment, in the autumn.

Dan Lasher, from the US Natural Resources Defense Council, countered immediately after the speech that the prime minister's proposal on UK emissions "doesn't respond to the threat she underscored". If developed countries merely freeze emissions, he said, global emissions will still increase with growing energy demand in developing countries.

The proposed British response fits within the loose guidelines on emissions laid down in the leaked draft report from working group III of the Intergovernmental Panel on Climate Change (see above). But other Western European countries plan reductions — West Germany has already committed itself to a 25 per cent reduction in emissions from 1987 levels by 2005.

Peter Aldhous

GLOBAL WARMING

A solution in iron solution?

Washington

A proposal that the greenhouse effect could be inexpensively mitigated by artificially stimulating massive blooms of algae in the Antarctic Ocean has outraged environmentalists and drawn attention from researchers, the press and even the White House. The proposal, by John Martin of the Moss Landing Marine Laboratory was first published in these pages (see *Nature* **345**, 114 & 156; 10 May 1990), but when the *Washington Post*, in a front-page story, independently picked up the idea last week, Martin's office was besieged by calls.

"I'm astounded", Martin says of the reaction. However, the National Research Council took his idea seriously at a workshop convened last December at the suggestion of Adam Heller of the University of Texas. During the two-day meeting, participants even suggested a $50 to $150 million trial experiment.

The logic of Martin's idea comes from evidence that the growth of phytoplankton in the Antarctic Ocean is restricted by a shortage of iron. Other nutrients abound, but in the southern regions far from dry land and wind-borne dust, Martin finds that levels of dissolved iron are among the lowest in the world. Because phytoplankton fix carbon dioxide during photosythesis, adding about 300,000 tonnes of iron to the ocean could stimulate algal growth sufficiently to soak up more than 2,000 million tonnes of carbon — roughly a third of the amount put in the atmosphere each year by deforestation and fossil-fuel burning.

For those who worry that enforcing drastic cuts in CO_2 emissions would return the developed world to the pre-industrial age, man-made algal blooms sound like an attractive way out. But Martin's solution to climate change is attracting plenty of criticism.

Biologists, noting the deadly effects of algal blooms in the Adriatic, are concerned about the impact on the food chain. Oceanographers and atmospheric scientists say that the phytoplankton may simply release most of the carbon back into the atmosphere when they die, rather than sinking to the ocean floor. And some policy analysts worry that if every industry could fill a tanker with iron to atone for its carbon sins, few would take expensive and restrictive measures to keep emissions down. "We're not iron-poor, we're pollution-rich", says Daniel Becker of the Sierra Club.

Environmentalists look poorly on 'megabiology' — global tampering with the biosphere. Atmospheric scientist Michael Oppenheimer of the Environmental Defense Fund is reminded of earlier suggestions to combat global warming with huge mirrors in space (to reflect away sunlight) or to regenerate stratospheric ozone with orbiting lasers. "The whole thing smacks of the mega-project-itis that infects large areas of the scientific community when it comes to environmental problems", he says. "If there is one thing we've learned in the last twenty years, it's that there are always unintended implications of what we do to the environment."

Reaction from the scientific community has been more encouraging, although serious questions remain unanswered. Among the more glaring gaps are the limited understanding of CO_2 exchange between the atmosphere and the ocean and the movement of carbon from the surface to greater depths. Although Martin says iron appears to be by far the most important limiting agent for algal growth in polar water, his is the only major study on the subject. When saturated with iron, large-scale phytoplankton blooms may still be limited by some other unexpected nutrient or condition. "Biological systems are famous for not doing what they're supposed to do", says David Caron of the Woods Hole Oceanographic Institution.

Others suggest that Martin may be observing an increase in the rate of algal growth, rather than its overall volume. But Martin says laboratory tests indicate that the addition of iron can increase total phytoplankton biomass tenfold. And even if it is only the rate of growth that is accelerated, short Antarctic summers put a premium on speedy development. During the few months when polar conditions are right, the fastest-growing algae are likely to produce the most biomass and soak up the most CO_2, he says.

Despite the doubts, the scientific consensus appears in favour of trying out Martin's theory in the field, on a modest scale. Martin suggests putting a tonne of iron in an area 10–100 km square in the Gulf of Alaska, which is relatively easy to get to and has phytoplankton conditions similar to the Antarctic. An experiment of that sort would cost between $10 and $50 million, he estimates.

Such a test could answer the most immediate questions about the impact on other plants and animals in the region of the algal blooms. The NRC is expected to convene another workshop on the subject in August or September. NRC officials say a full-scale, peer-reviewed report could be released in late 1991. Along with the National Science Foundation and the Electric Power Research Institute, which funded part of the NRC workshop, the US Environmental Protection Agency has expressed interest in funding further research in the area as part of the $1,000 million US climate change programme.

G. Christopher Anderson

FRENCH UNIVERSITY REFORMS

Emergency investment plan under way

Paris

A CABINET meeting on 23 May approved French education minister Lionel Jospin's plans to invest an extra FF16,000 million ($2,900 million) over five years in order to relieve severe and growing overcrowding in France's universities (see *Nature* 342, 723; 1989).

The move follows a promise by President François Mitterrand earlier this month to build 1.5 million square metres of new university facilities. Mitterrand said last week that the new plans are "unprecedented", but added that so is the demand Student applications for university places were up by about 80,000 last year and the extra demand is expected to be even greater this autumn. Early last year, Jospin declared that 80 per cent of pupils should leave school with a university entrance certificate *(baccalauréat)*.

University buildings in the Paris region are already severely overcrowded and several are showing signs that they were built in a hurry to meet a similar expansion in the early 1970s. Five new universities are planned for suburbs and dormitory towns around Paris, another for Pas-de-Calais on the Channel coast and possibly two others in the provinces.

There is also a critical need to extend existing facilities, especially libraries and student accommodation. But the government's new package is unlikely to b[illegible] sufficient to cover all the costs. So Jospin i[illegible] relying upon regional and local governments to share some of the burden. This is may not be easy, especially since the government is unwilling to let local authorities have much of a say in higher education policy.

The plans have received a lukewarm reception from university teachers' unions. The new funds are felt undeniably to be welcome but are inadequate, they say. Also they fear that local governments will not pick up the extra bill because they will be reluctant to run the electoral risk of having to pass it on to the taxpayer.

Jospin's plans are still not out of the woods. The education budget has to be approved by parliament in the autumn, although the proposals have received guarded opposition support. A series of hearings are also planned this summer to negotiate the university reforms in each region and administrative department of the country.

Peter Coles

SPACE ASTRONOMY

High hopes for Astro-1

Washington

IN 1978, when the space shuttle was being advertised as a regular bus service into Earth orbit, the National Aeronautics and Space Administration (NASA) invited scientists to propose instrumental payloads that could be flown repeatedly, once or twice a year, making the shuttle into an easily used space science platform. But it has taken 12 years for the first of those proposals, a set of astronomical telescopes known as Astro-1, to get off the ground. And the future of Astro, due to have been launched yesterday, 30 May, is far from guaranteed. No additional missions are scheduled, and only after this first flight has been evaluated will NASA decide whether the instruments, tailor-made for the shuttle, will be used again.

Astro-1 consists of two separate astronomical packages which find themselves on the same flight because of the disruption caused by the 1986 Challenger explosion. The larger part of Astro-1 is a set of three ultraviolet telescopes — an imager, a spectrophotometer and a polarimeter — that was to have flown in early 1986, along with a camera specially designed to photograph Comet Halley. When shuttle flights resumed three years later, Halley was long gone, and instead of the camera the Broad-Band X-ray Telescope (BBXRT) was mounted alongside. BBXRT was originally part of an X-ray astronomy package, but was pulled out and made ready earlier in order to take X-ray pictures of supernova 1987A.

The two parts of the Astro-1 package are on separately steerable mounts attached to pallets inside the cargo bay. As the shuttle pilot holds the craft for several hours in a fixed attitude, other crew members point the telescopes at a series of predetermined target stars. All three ultraviolet instruments — the Hopkins Ultraviolet Telescope (HUT), the Ultraviolet Imaging Telescope (UIT) and the Wisconsin Ultraviolet Photo-Polarimeter Experiment (WUPPE) — are aligned together and observe the same object, and for about 20 per cent of the planned observations BBXRT will also be pointed in the same direction to provide simultaneous X-ray imaging. Both ultraviolet and X-ray astronomy specialize in energetic cosmic phenomena: young stars, supernova remnants, quasars and hot interstellar gas.

With the exception of UIT, which takes photographs that will be developed on the ground, the Astro-1 instruments will send down data in real-time, allowing astronomers at Marshall Space Flight Center in Alabama to re-adjust the observing program should some transient phenomenon such as an X-ray binary or a cataclysmic variable erupt. According to Peter Serlemitsos, BBXRT project investigator, the enforced hiatus caused by the Challenger accident has allowed mission controllers to become much more comfortable with last-minute schedule changes.

In the weeks following the ten-day mission, astronomers will be more than usually anxious to see what their search of the sky has trawled up. Although NASA has promised money to support maintenance and even some further development of the Astro-1 hardware, according to HUT co-investigator Knox Long, no commitment even in principle to another launch has been made. Both Long and Serlemitsos are expecting NASA to evaluate Astro-1 later this year, with the payloads being kept in storage at the Kennedy Space Center until, as Long puts it, "the situation clarifies". Another mission could be put together at about six months' notice, provided everything works as planned, but the nearest hint of an open slot in the shuttle manifest, says Serlemitsos, is not until 1993.

The possibility of avoiding these problems by making the two parts of the Astro mission into self-contained satellite observatories, needing the shuttle neither for launch nor for operation, is now almost gone. Astro-1 may have to do spectacularly well to avoid premature consignment to a museum.

David Lindley

SUPERCONDUCTING SUPER COLLIDER

Pot of gold sought in the East

Washington

ARMED with little more than a letter from President Bush and a blueprint, a US Department of Energy (DOE) team departs for Japan and Korea this week to find partners for the Superconducting Super Collider (SSC).

Although DOE officials do not expect to come back with any contracts, they do hope to start work on government-level collaboration agreements. However, what DOE is willing to offer in exchange for money or equipment remains a 'classified' secret. Some Japanese researchers have expressed interest in the project, but the government of Japan has sent few public signals of encouragement.

DOE officials have previously indicated that the international foray would begin as soon as a final cost estimate for the 20 TeV proton accelerator had been set. But as DOE deputy secretary W. Henson Moore and other DOE and White House officials prepared to leave last week, that price was still only a guess of around $8,000 million.

Congress recently passed legislation demanding that one-third of the project's cost come from non-federal sources. The state of Texas has promised $1,000 million, leaving DOE still to find an additional $1,600 million or so elsewhere. So far, the only definite international promise had been $50 million from India, but even that appears to have gone soft. "We are not sure how solid any of those past commitments are. We're treating them all as tentative", Moore said.

Whether DOE will allow foreign partners to make the more sophisticated components of the accelerator — to the advantage of their high-tech industries — remains an open question. Congress has stressed that technological manufacturing, particularly of the SSC's superconducting magnets, should remain at home (for some politicians, technology spin-offs remain the project's chief selling point). Moore announced that the contract for the magnets is now up for grabs, and both US and international companies may apply. But whether Japan will be given a real chance at the contract as part of a collaboration deal is not known.

DOE officials plan to extend their search for partners to Europe in October. By then they also hope to have named a director for the project at DOE headquarters and to have settled on a final price.

G. Christopher Anderson

Tokyo

Yubun Narita, director of scientific affairs at the Japanese Ministry of Foreign Affairs, says that the meeting will be the first detailed explanation of the project by US government officials, and as such Japan cannot make any commitments at this stage. Only after hearing the US explanations and requests for help will Japan decide what (if anything) to do next.

The views of Japanese high-energy physicists on the SSC are divided. Some researchers at the High Energy Physics Laboratory (KEK) in Tsukuba are keen to participate in the experimental phase of the programme. But others in the Universities would prefer to collaborate with the European Laboratory for Particle Physics (CERN) in the operation of CERN's proposed Large Hadron Collider because participation in that (if it goes ahead) is expected to be cheaper.

At present, there seems to be little support among the Japanese scientific community for Japan to contribute to the construction costs of the SSC. One physicist at KEK asks "What is the point if they don't want our superconducting magnets? Would our contribution be some form of ODA (Overseas Development Assistance)?"

David Swinbanks

AIDS RESEARCH

Protesters ask for more

Washington

Two hundred police officers wearing riot helmets and rubber gloves were called in to protect the US National Institutes of Health (NIH) last week as a crowd of nearly 1,000 activists massed on its campus demanding an acceleration in AIDS research, increased access to experimental drugs for minority groups and more vigorous research into AIDS-related opportunistic infections.

About 60 demonstrators were arrested on the campus for attempting to break into buildings and a further 21 were arrested while staging a sit-in in the office of Daniel Hoth, director of the division of AIDS for the National Institute of Allergy and Infectious Diseases (NIAID). But despite fervid chants of "We want a cure, this is a war" and "storm the NIH", there were no reports of serious injuries or damage to property, with most demonstrators being content to wave placards or adorn NIH trees with symbolic red tape.

Anthony Fauci, director of NIAID, described the protest as "street theatre" — interesting, but unhelpful. "It was rather inappropriate that they should demonstrate against the very institution that is trying to help them", he said.

Organized by the militant US AIDS activist organization ACT-UP, the demonstration marks the latest battle in an increasingly emotional campaign by activists to influence the course of AIDS research. Mark Harrington, ACT-UP's spokesman on research policy, claims that the demonstration was precipitated by a growing feeling among activists — that NIH policies on drug development are "too conservative and unimaginative", and that there are "too many drugs coming out of the test-tube but not getting into bodies". The last straw, says Harrington, was hearing that the initiation of new clinical trials of drugs would be delayed by nine months because the database was switched from the Research Triangle Institute at North Carolina to the Statistical and Data Analysis Center at Harvard Medical School.

But Fauci dismisses that claim as one of a number of "absolutely untrue" allegations — "the same rate and pace and number of trial protocols have been put into effect" this year as were executed last year, he says.

NIAID's policies on research and clinical trials have been bitterly attacked in documents released both independently by ACT-UP and jointly by 25 different AIDS activist organizations, ACT-UP among them. One of the key criticisms is that NIAID's clinical trials, which at present enrol only 1 per cent of US AIDS patients, are too limited and exclude minority groups such as children and pregnant women. Fauci, however, says that the activists fail to acknowledge that the need for increased medical services for minority groups in the United States is part of a "general problem in the medical-care delivery system". He argues that NIAID has already introduced a host of measures designed to help increase the accessibility of clinical trials to minority groups and points out that 15 of its trial units are now devoted to paediatrics.

The documents also claim that NIH-directed research into the many opportunistic infections that afflict AIDS patients is inadequate, particularly as some AIDS patients are now living longer because of AZT. Fauci says that NIAID is "clearly aware of the fact that opportunistic infection research has not proceeded as quickly and as intensively as research on HIV anti-retroviral drugs" and has already taken steps to "correct that" by expanding its trials of treatments for opportunistic infections. The number of patients involved in such trials has more than tripled in the past year, he says.

The other main allegation is that in setting their research priorities some NIH researchers are being swayed by the need to please the drug companies that top up their coffers. The activist documents demand that principal AIDS researchers be bound to disclose their financial ties just as members of FDA advisory committees are. But Fauci says the demand is unnecessary — AIDS researchers must already divulge their financial interests, and the staff of NIAID, who ultimately decide which drugs should be tested, are forbidden to have any ties to industry.

David Concar

BSE

One down, one to go

London

THE UK agriculture ministry has announced that a 15-month-old cow suspected of having bovine spongiform encephalopathy (see *Nature* 345, 280; 24 May 1990) did not have the disease. But the ministry says that its Central Veterinary Laboratory at Weybridge is still examining a second suspected case in a cow born after the ban on cattle feed containing ruminant tissues.

Peter Aldhous

EC SCIENCE

Worries over Framework

London & Munich

THE emergence of the European Communities' (EC) third Framework research and development programme last month, with a shift towards more basic science, has provoked apprehension in the UK research councils that British research will suffer as resources switch to EC projects. The new Framework Programme is set to run from 1990 to 1994 and has a budget of 5,700 million ECU (1 ECU = £0.72), an increase of over 300 million ECU over the previous five-year programme. But very little of the new programme's budget will be spent until 1991, and the previous programme runs into 1992.

In Britain, the Treasury adjusts the budgets of spending ministries to account for the United Kingdom's contributions to (and returns from) the EC. This 'attribution' depends on secret negotiations before the annual autumn statement on public expenditure. Similar arrangements exist in other member states, including West Germany, but they are less rigid. "Our contributions to the EC have been growing for the past 10 years and BMFT [research ministry] budgets have never been cut", says a BMFT spokesman.

'Attribution' worries the UK research councils because as the new Framework programme increases support for basic research, the Department of Education and Science (DES)'s budget, from which research councils are funded, may be squeezed. A total of 518 million ECU is planned for basic science under the new Framework programme, increased from 180 million ECU spent from 1988–92.

A Science and Engineering Research Council (SERC) memorandum to the House of Commons Education, Science and Arts Committee says that it is difficult for SERC to assess EC research programmes "without knowing whether resources . . . would come from the [DES] science budget". Dai Rees, secretary of the Medical Research Council, fears that the DES 'flexibility margin' — a fund of about 15 million ECU kept back for priority research arising after the rest of the science budget is allocated — may be swallowed up by attribution against EC research spending. Rees is critical of EC medical science, also set to grow under the new Framework programme. A tendency to "throw money at things to look good" exists in the EC, he says, "which goes against the grain when you're turning down good projects".

Sir Peter Swinnerton-Dyer, chairman of CODEST, the committee that oversees EC support for basic science, says Rees's concern over attribution against the DES budget is valid "in principle". But he thinks Rees overestimates the problem. Eileen Buttle, secretary of the Natural Environment Research Council, says the research councils must ensure that their views are heard when EC research programmes are drawn up.

Peter Aldhous & Steven Dickman

GENETIC ENGINEERING REGULATION

Superpigs go to market

Sydney

The Australian government is under pressure to introduce new laws to govern the regulation of genetic engineering after a public outcry over the sale for human consumption of 53 genetically altered pigs. The pigs had come from a research programme aimed at breeding 'superpigs' through the introduction of extra copies of the gene for porcine growth hormone. They were sold for slaughter apparently without approval from any of the bodies regulating genetic engineering.

At present, guidelines on genetic engineering in Australia are issued by the federal government's Genetic Manipulation Advisory Committee (GMAC). Any organization seeking to release a genetically altered organism into the environment is expected to seek advice from GMAC, although compliance with the guidelines is voluntary.

Metrotec Pty Ltd was responsible for the experiments with the pigs. The 53 pigs they sent to the slaughterhouse were the offspring of a transgenic boar not expressing the gene and had been removed from the programme because their growth hormone gene was not functional. The company is part owned by the University of Adelaide, but the university's Institutional Biosafety Committee had not assessed the dangers of releasing the pigs.

Barry Lloyd, managing director of Metrotec, said on ABC Radio that while the university is required to conform to the guidelines on genetic engineering, private companies are not.

Metrotec further argues that the pigs were released with the knowledge of both state and federal government bodies. John Smeaton, a director of Metrotec, says that the National Health and Medical Research Council and the South Australian Health Commission had approved the meat for consumption. Smeaton also says that the release of the pigs had been discussed with Brian Booth, a representative of the Recombinant DNA Monitoring Committee, the precursor to GMAC.

According to Lorne Skene, project manager of the genetic manipulation section of the Victorian Law Reform Commission, GMAC has no powers of direct sanction should their guidelines be ignored. GMAC can, however, recommend the removal of federal funding and tax incentives from both the research group involved and the parent institution.

The Australian Conservation Foundation (ACF) is taking advantage of the questions raised by release of the pigs to push for a national uniform system of legal regulation. Phillip Toyne, director of ACF, said: "As companies increasingly seek to commercialize research results new laws are becoming even more necessary." The University of Adelaide also believes GMAC guidelines should become enshrined in legislation. "This would ensure that all organizations operating in this area would be required to meed defined standards", the university registrar said in a public statement.

In a report released last year, the Victorian Law Reform Commission recommended legislation requiring mandatory notification of any proposed release of a genetically altered organism to GMAC and to any relevant state or federal body, such as the Department of Agriculture.

The commission also called for an environmental assessment before the release of any experimental organisms and the advertising of proposed releases "to ensure that interested people can obtain information and participate in decision-making before the proposal is approved". Australia's 'superpigs' were the world's first transgenic animals with enhanced production characteristics. In a project run by Bob Seamark, reader in endocrinology at the University of Adelaide, transgenic pigs incorporating genes for porcine growth hormone grew as much as 17 per cent faster, with a 30 per cent better feed conversion rate.

Seamark says he is against the introduction of legislation, "because it is inflexible and irreversible, [and] could close down the whole of recombinant research in one act of parliament. The solution is to develop confidence in researchers who abide by voluntary guidelines. They are not a bunch of naive wackers doing strange and evil things." Metrotec, however, would welcome legislation. "We would be happy with a stable set of rules to which we would have some input. We don't want to invest five years work into these animals only to have someone reinterpret the voluntary quidelines and say we can't market the pigs", Smeaton says.

Tania Ewing

Correction: Dounreay

Thomas Robertson of the Hahn Meitner Institute (HMI) was misquoted in the article "Waste issue threatens reactor" on 10 May (*Nature* **345**, 105; 1990) as saying that fuel from a West Berlin research reactor is not kept separate from nuclear bomb material at the Dounreay facility in Britain; HMI has since been assured that spent fuel from HMI would not be reused for military purposes at Dounreay. ☐

Correction: *In vitro* fertilization:

An editing error in last week's news item on an MRC study on IVF (*Nature* **345**, 283; 1990) changed the meaning of the last sentence, which should read: "In the opinion of Brian Lieberman, from St Mary's Hospital in Manchester, improved preservation of frozen embryos over the next two or three years should mean that multiple births will be *less* common . . .". ☐

SOVIET SCIENCE

Gorbachev leads silicon summit

Washington

Soviet President Mikhail Gorbachev and other Soviet officials will visit Stanford University and research centres in Silicon Valley in California next week to lay the groundwork for a similar science and high-technology oasis near Moscow. The visit will begin a ten-day meeting between the two countries to establish trade relations, with the aim of encouraging US companies to participate in a 'Soviet Silicon Valley' in the city of Troitsk, near Moscow.

Known as the Soviet Silicon Summit, the meeting will follow the Bush–Gorbachev summit at which the United States is expected to grant the Soviet Union 'most favored nation' status. US officials have also declared their intention to lift many of the restrictions imposed by the Coordinating Committee on Multilateral Export Controls (COCOM) on the sale of high-technology products to the Soviet Union.

Although Gorbachev is expected to spend only one day at Stanford, visiting the university and meeting Silicon Valley business leaders, other Soviet officials will stay to tour the area's laboratories and production facilities. Next week, the officials are expected to unveil plans to convert Soviet military technologies to commercial use in fields such as microelectronics and communications.

As part of their drive to beat swords into microchips, Soviet officials have recently signed an agreement with Bechtel International, a large US engineering company, to study the feasibility of developing a 'technopolis' near Troitsk, which is already home to the Kurchatov Institute of Atomic Energy. The Troitsk Research Development Project aims to attract international businesses to help develop and invest in Soviet advances in fields such as nuclear fusion and laser technology.

The Soviet Union is expected to spend about $100 million a year to support the project. If plans are approved, Bechtel will eventually build conference centres, a new international airport and an advanced telecommunications network in Troitsk by the year 2020. By that time, Troitsk, which at present has a population of 30,000, is expected to have a population of over 100,000.

During the Silicon Valley summit, to be held from 4 to 14 June in Santa Clara, California, Soviets will meet US companies interested in a range of business connections. The 15-member Soviet delegation is the largest to visit the United States to forge ties with US high-technology companies. After the meeting, the Soviets will open a full-time Silicon Valley sales office featuring demonstrations of Soviet software, Soviet market research data and a research library.

G. Christopher Anderson

TSUKUBA SCIENCE CITY

Success coming — but only slowly

Tsukuba

A RUSH by private companies to build research institutes is finally bringing Tsukuba science city to life after nearly two decades when it seemed Tsukuba might never amount to anything more than handful of government research institutes spread out over a 20 km strip of paddy fields.

New statistics from a Tsukuba city office show that since 1985 the number of private research laboratories has more than tripled from fewer than 30 to more than 90. Growth has been particularly dramatic in the past year: 18 new institutes opened in 1989, many more are under construction or planned and the number of company researchers and their support staff has risen dramatically to 5,000 — close to the number (6,700) of government researchers.

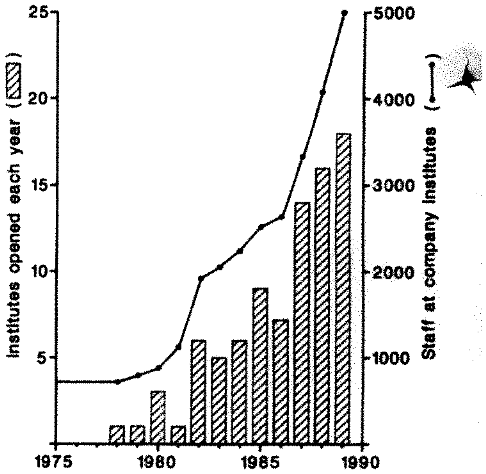
The newcomers include giant pharmaceutical and chemical companies, such as Yamanouchi Pharmaceutical, Sankyo, Takeda Pharmaceutical, Kyowa Hakko, and Sumitomo Chemical, and electronic manufacturers such as NEC. Some of the biggest laboratories belong to foreign companies (see opposite). And although much of the research is product-orientated, there has also been a blossoming of company institutes devoted to much more basic research (see below). Around 50 small- to medium-sized factories producing high-technology products have also come in with the wave of private investment.

The surge of investment is good news for the government, which has spent more than a million million yen on a city in which until recently few people wanted to live. Plans to establish a science city in Tsukuba were hatched in the 1960s when many government research institutes located in Tokyo were rundown and overcrowded and expansion was impossible because of skyrocketing land prices. Hence came the idea to make a mass move to a new site outside Tokyo. The student riots of the late 1960s and early 1970s also accelerated moves to establish a new university in Tsukuba.

By the early 1980s, 46 government research institutes and two universities had moved to the new site. But they were spread out over a vast area. The 'city' lacked a shopping centre and cultural facilities. The nearest railway station was a 20-minute bus or expensive taxi ride away. And the local country schools could not meet the exacting standards of academic parents. The result was that many researchers chose to spend four hours a day commuting from Tokyo rather than live in Tsukuba.

The situation is rapidly changing now. The influx of private researchers and their families helped to boost the population of Tsukuba city from 127,000 in 1985 to over 140,000 last year (the population is more than 174,000 if one includes outlying areas of the science city). New schools have been established to cater for the incomers. And although Tsukuba still has vast tracts of farm land, there is a shortage of land for housing — Japan's tax system encourages farmers to hold onto their farms.

Land prices have more than quadrupled in the past four years to over ¥1,000,000 ($6,250) per tsubo (3.3 square metres) forcing some poorly paid researchers to set up house in country villages an hour's travel away. Many of the government researchers who previously scorned living in Tsukuba are now looking for land and this has also helped drive up the prices.



The histogram shows the number of private company research institutes opened each year in Tsukuba, and the graph shows the population of private company researchers and support staff in the city.

So why the sudden rush to Tsukuba? The government-sponsored Tsukuba Science Expo (see *Nature* **314**, 213–220; 1985) held in 1985 at a cost of ¥210,000 million ($1,300 million at current exchange rate) acted as a great spur to private investment. Large department stores and hotels were built to cater for the 20 million visitors to EXPO, helping make Tsukuba a more liveable place. Also several of the companies have built their laboratories on the former EXPO site where cheap land was available.

Company officials cite two main reasons for their move to Tsukuba: availability of large tracts of cheap land not far from Tokyo, and access to facilities and information at government institutes. The synchrotron radiation source at the High Energy Physics Laboratory has many

Freeing the imagination

NEC'S new laboratory in Tsukuba provides an insight into the fundamental changes that are going on in the research and development policies of major Japanese companies. The laboratory is vast by Japanese standards, providing an average of 100 square metres of space for each of the 160 staff plus all of the latest equipment. But more surprising is that much of the research going on there is not of immediate use to a major electronics and computer manufacturer.

Researcher Shigeru Tanaka, for example, is trying to understand how the brains of cats and monkeys process visual information using a mathematical model that describes the domain structure of magnetic thin films. (Tanaka sees striking similarities between the patterns of up-and-down magnetization in thin films and the stripes of occular dominance of the right and left eye in the visual cortex of the brain.) Toshikazu Takada spends his time simulating collisions between hydrogen molecules on a supercomputer. And another researcher plans to use pulsed lasers to investigate chemical reactions.

Such basic research is something completely new at a Japanese company laboratory. Hisatsune Watanabe, general manager of the laboratory, says that he sometimes has difficulty explaining to production managers the relevance of what is going on in Tsukuba. But the upper echelons of NEC are committed to backing basic research.

Until recently, NEC had only one research laboratory, in Kawasaki, not far from Tokyo. But now the company has five. A laboratory adjacent to a semiconductor plant in nearby Sagamihara acts as a 'liaison office' between the research and development sections of NEC and production. The new laboratory in Tsukuba and another in Princeton 'New Jersey' in the United States, on the other hand, are devoted to much more basic research. A fifth laboratory for computer technology, the NEC Kansai C&C Research Laboratory, has just opened in Osaka.

Behind these moves to establish separate laboratories lies a deliberate strategy to create the freedom for more creative research by physically separating the people involved in production from those involved in basic research. Ironically, this is quite the opposite of what is going on in the United Kingdom and other Western countries, where government policy aims at bringing academics into closer contact with industry. D.S.

Famous foreign faces

Among the wave of new companies coming to Tsukuba are several well-known foreign names, including the giant pharmaceutical and chemical concerns Glaxo, ICI and Upjohn, and the US semiconductor manufacturers Intel and Texas Instruments.

Upjohn's huge laboratory with 170 researchers was completed in 1988 at a cost of ¥14,000 million ($87 million). A spokesman says that the decision to a set up in Tsukuba is part of a global strategy to have research centres in the three key markets of the world: the United States, Japan and Europe.

Glaxo gives similar reasons for spending ¥20,000 million on a laboratory that will open in 1992 with a planned staff of 250 researchers.

At present, about 70 per cent of Upjohn's efforts are devoted to the clinical development of drugs for the Japanese market, which is second in size only to that of the United States.

But in the future the institute intends to devote more effort to the development of new drugs for markets throughout the world by "tapping into the rapidly expanding resource of Japanese pharmaceutical science".

Similarly, Texas Instruments intends to expend much of its efforts on basic research when its opens an institute for semiconductor research next year with an initial complement of 100 to 150 researchers drawn mainly from Japan. D.S.

industrial users, for example, and there are over 80 'research clubs' many of which are centred on Tsukuba University.

In addition, the local Ibaraki prefectural government, the Japan Development Bank, and 74 companies last year established the Tsukuba Research Support Centre, with a fund of ¥28,000 million ($175 million) to provide companies and researchers with information on the latest developments in the science city. But while information networks may be well-developed, Tsukuba's transportation system remains grim. The only direct route to Tokyo is by bus which takes just over an hour, provided there are no traffic jams and a seat is available. Local government officials promise a direct train line to Tokyo within the next ten years, and there are also plans to build a highway in a huge loop around Tokyo passing near Tsukuba and joining the ends of the yet-to-be-built Tokyo Bay bridge.

If either plan is realized, Tsukuba can expect another surge in development and as one researcher put it "I can sell my house and retire on the proceeds".

David Swinbanks

ROMANIAN SCIENCE

Back to the future

Munich

In a bid to encourage the rebirth of culture and science, researchers and professional people have reestablished Romania's Atheneum Society, a scientific academy banned in 1947 by the Communists.

The refounding of the society is important because the Romanian Academy is now discredited. The founders believe that only by starting a new and separate organization can science and culture be restored to their proper place in Romanian society. But vice president Ion Mircea Enescu, an architect, emphasizes that the society is not intended to replace the academy.

The original Atheneum Society was founded in 1865 as a cultural and learned society with 33 sections, from biology, law, fine arts and medicine to architecture and "fundamental science" — physics, mathematics, geometry and philosophy. It will be reconstituted in the same form, with the number of members fixed at 151.

Significantly, the Culture Ministry in the interim government of Ion Iliescu has offered its support to the society, but the amount is not yet clear. The society hopes to receive support through 'subscriptions' sold in Romania and abroad.

"We want only professionals" for the new society, says Enescu. The society, which has already nominated 100 members, will select only people considered politically "beyond reproach", he says. A very few were in the Communist Party but none were "professionally involved". None of the current academy members have been asked to join.

Another difference is the age of the members. Enescu says that the Atheneum Society will nominate only people who are still active in their fields. The current nominees are between the ages of 45 and 70. The average age of academy members is over 75 years.

At first, the society will sponsor public lectures and conferences on topics of current relevance such as ecology and the "philosophy of architecture and construction in Romania", says Enescu. The society may also advise the government in some capacity, though "we don't yet know the mechanism for this", he says.

Enescu is hopeful that the society will receive political as well as financial support from the Iliescu government, even though he pronounces the 20 May election that swept Iliescu to the presidency "a fake".

Steven Dickman

AIDS

One finger in the Romanian dike

Munich

A new report from the Romanian government suggests that efforts by it and foreign organizations are failing to stop the spread of AIDS in Romania. The report reveals that there were 478 cases of clinical AIDS in Romania as at 1 May, far more than the earlier estimates of 100 cases.

Even the new figure may be a drastic underestimate of the true situation. It is not known how many people are infected with HIV, the virus that causes AIDS.

Most alarming, 428 of the cases were found in children less than 13 years old. Of these 424 are under four. The now discontinued practice of giving 'microtransfusions' of blood to malnourished infants, many of them orphans, is thought to be behind the explosive spread of AIDS (see *Nature* 343, 579; 1990).

The blood supply, and the needles and syringes used, were contaminated by the AIDS virus. In a random nationwide sample of blood taken from children in orphanages, nearly ten per cent of the children tested positive for HIV, although the World Health Organization (WHO), based in Geneva, warns that this number might be high because of double reporting.

Testing of blood donors (there are an estimated 600,000 in Romania annually) and of blood already stored in blood banks is not being carried out systematically, partly because of a shortage of testing equipment. Disorganization in the Health Ministry is also to blame.

Foreign relief organizations have delivered modern test equipment as well as thousands of sterile syringes and needles for injections and transfusions. The French organization Medicins du Monde alone has provided three ELISA (enzyme-linked immunosorbent assay) readers and thousands of test kits to laboratories in Bucharest for AIDS testing. But the Health Ministry has begun testing blood only in Bucharest and the three hard-hit eastern cities of Constanta, Iasi and Giurgiu. At least 20 – 25 laboratories including readers and kits will be required before blood screening can be comprehensive.

Romania's AIDS troubles may have only begun. Testing blood is a relatively simple job, says epidemiologist David Heymann of WHO, compared to the monumental task of educating the population about AIDS. According to Heymann, the Romanian press is just beginning to tackle the job. The regime of late dictator Nicolae Ceaucescu, which was overthrown in a bloody revolution in December 1989, had always claimed that AIDS did not exist in Romania and even forbade physicians to discuss it publicly.

Steven Dickman

Problems of tenure in Japan

SIR—A recent Commentary (*Nature* **340**, 337; 1989) noted that "in practice it is very difficult if not outright impossible for a foreign national to get tenure" in Japan, but discussed neither the cause of this difficulty nor how it could be ameliorated. The small number of tenured foreign faculty and the outright refusal by most universities to grant tenure to foreign faculty are particularly incongruous in the light of a recent proposal by Tokyo University "to create a science faculty which is also an internationally connected research community" (see *Nature* **338**, 99; 1989). One of the goals of Tokyo University's reform plan is to create an "intellectual climate that will be attractive to people from overseas". However, the "people from overseas" will be temporary employees, mostly at the postdoctoral level, and will not be encouraged to apply for permanent positions. It is questionable whether such a plan should be characterized as "internationalization". Even on a purely pragmatic level, why does anyone think top foreign scientists will be interested in working in temporary posts in a far-away country with an unfamiliar language, where the only available career path is getting the boot?

I have been an associate professor in the department of geophysics of the faculty of science of Tokyo University since August 1984. My being hired as a tenured faculty member was due to the extraordinary efforts of Professor M. Kumazawa and his colleagues, who were committed to a policy of non-discrimination in hiring foreign faculty. My experience has been, on the whole, extremely positive from both a personal and scientific point of view.

Unfortunately, however, almost all foreign faculty members (that is, regular academic staff, excluding research associates) of Japanese national universities (including, hereafter, research institutes and joint-use institutes of *Monbusho*, the Ministry of Education, Science and Culture) are appointed for terms of one to ten years, with the possibility (but not a guarantee) of reappointment, whereas their Japanese colleagues are tenured. Temporary foreign faculty members, who are only nationally equal to their tenured Japanese colleagues, cannot readily become part of the Japanese scientific structure. Students, who require professorial assistance in finding employment, are unlikely to regard a temporary foreign professor as a desirable adviser. Finally, the temporary foreign staff cannot safely dispute the views of the tenured Japanese faculty who will decide on their contract renewals.

As of 1 July 1989 (statistics provided by *Monbusho*) Japanese national universities employed a total of seven tenured foreign faculty members, but there were also 113 untenured foreign faculty members, with an average contract term of 2 years and 10 months. The following table summarizes the most recently available employment statistics. Note that all Japanese nationals are tenured, while almost all foreign faculty are temporary.

	Foreign nationals		Japanese nationals	
Rank	Tenured	Temporary	Tenured	Temporary
Professor	2	21	16,132	0
Assoc. profs.	4	52	14,276	0
Lecturers	1	40	5,216	0
Totals	7	113	35,624	0

Japanese law was changed in 1982 to permit the employment of foreign nationals as faculty members at Japanese national universities. The regulations issued by *Monbusho* to implement the new law state in part (my translation) that "in principle foreign faculty should be hired on a temporary basis, but, in unavoidable circumstances", the granting of tenure to foreign faculty "is not illegal". (The cognizant governing body of each university makes the final decision on what constitutes an unavoidable circumstances.) However, only five universities (Tohoku, Tokyo, Niigata, Kyushu and the National Laboratory for High Energy Physics) use this clause to employ tenured foreign faculty members.

The extremely small number of tenured foreign faculty members at Japanese national universities is thus primarily due to the rules and personnel decisions of individual national universities, rather than to Japanese law or *Monbusho* regulations. Why do almost all Japanese universities refuse to grant tenure to foreign faculty? Why do the handful of universities that will grant tenure to foreign faculty do so only in a small number of cases? And what, if anything, can be done to improve the situation?

First, no matter how much the administrators of Japanese national universities talk about internationalization, they have not truly grasped the notion that the development of an open university requires fully equal treatment of foreign and Japanese faculty. Second, because there is excessive concern over what would happen if a tenured foreign faculty member proves unsatisfactory, administrators prefer to retain control by hiring foreign faculty on a temporary basis. This eliminates the risks (both real and perceived) of hiring tenured foreign faculty, but also eliminates the possible benefits, which, it seems to me, far outweigh the risks. In any case, problems involving tenured Japanese nationals are by no means unknown.

Finally, it should be noted that Japanese universities are not fully open even on a national level. For example, in 1987, 86.8 per cent of the academic staff of Tokyo University were graduates of Tokyo University (*AERA*, No. 15, p. 15; 1989); the existence of such inbreeding is inconceivable at, say, any of the US universities. The failure of Japanese national universities to grant tenure to foreign faculty is only one aspect, then, of their general lack of openness, a theme I have discussed at length elsewhere (*Kagaku* **56**, 566; 1986). Further opening of Japanese universities to foreign faculty should also lead to a great increase in the movement of personnel between universities within Japan.

Unless the present discriminatory tenure policies are scrapped, I believe that the internationalization of Japanese higher education is doomed to fail, to the detriment of all concerned.

ROBERT J. GELLER

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Tokyo, Japan

Views of the staff

SIR—In "The museum director's view . . ." (*Nature* **345**, 198; 1990) the director of the Natural History Museum, Dr Neil Chalmers, states "we have chosen to concentrate on those areas of taxonomic research where we already have the greatest strength. . . . We have identified six such areas . . .". His use of "we" in this context could imply that the museum scientists took some corporate decision to alter the direction of research at the Natural History Museum.

Nothing could be further from the truth. The decisions were taken by a group of seven; the director, associate director and five heads of science departments, few of whom are involved in collection based research. The remainder of the museum's 300 scientists were not consulted.

Such is the sense of outrage at the museum that our senior scientists have volunteered to set up a committee, working with their trade union, IPMS, to defend science at the Natural History Museum, the museum staff are experts in the field of taxonomy, which the director is not. They are very clear about where the science priorities lie, and it is tragic that the director excluded this body of expertise from development of the corporate plan — a wholly avoidable situation.

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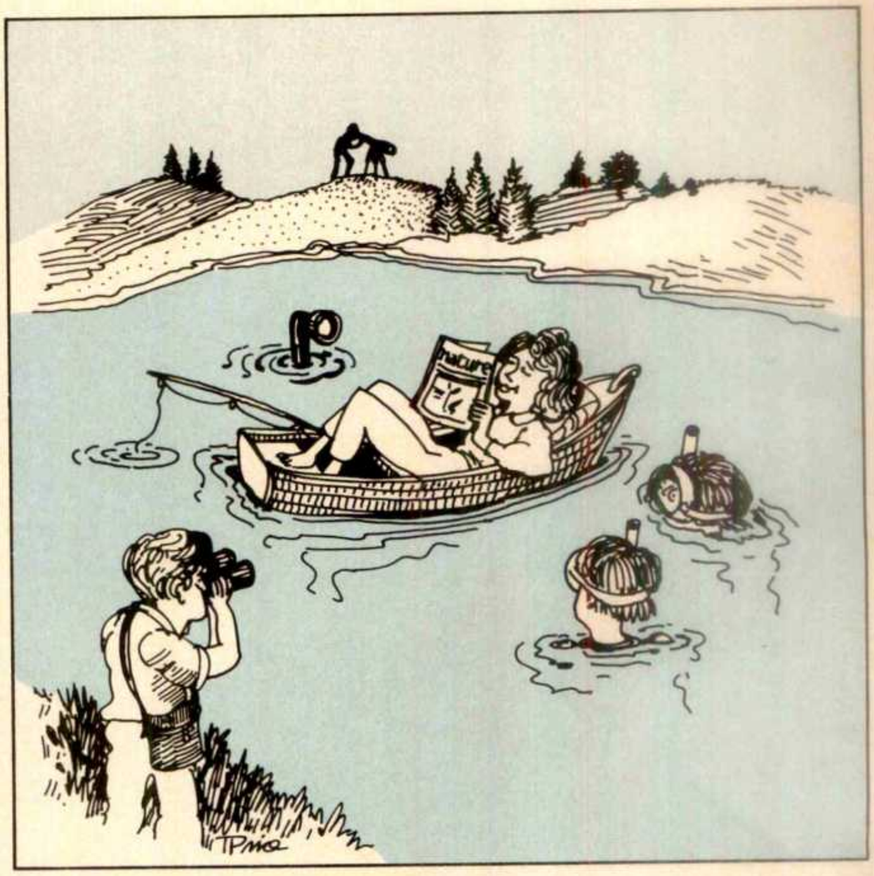

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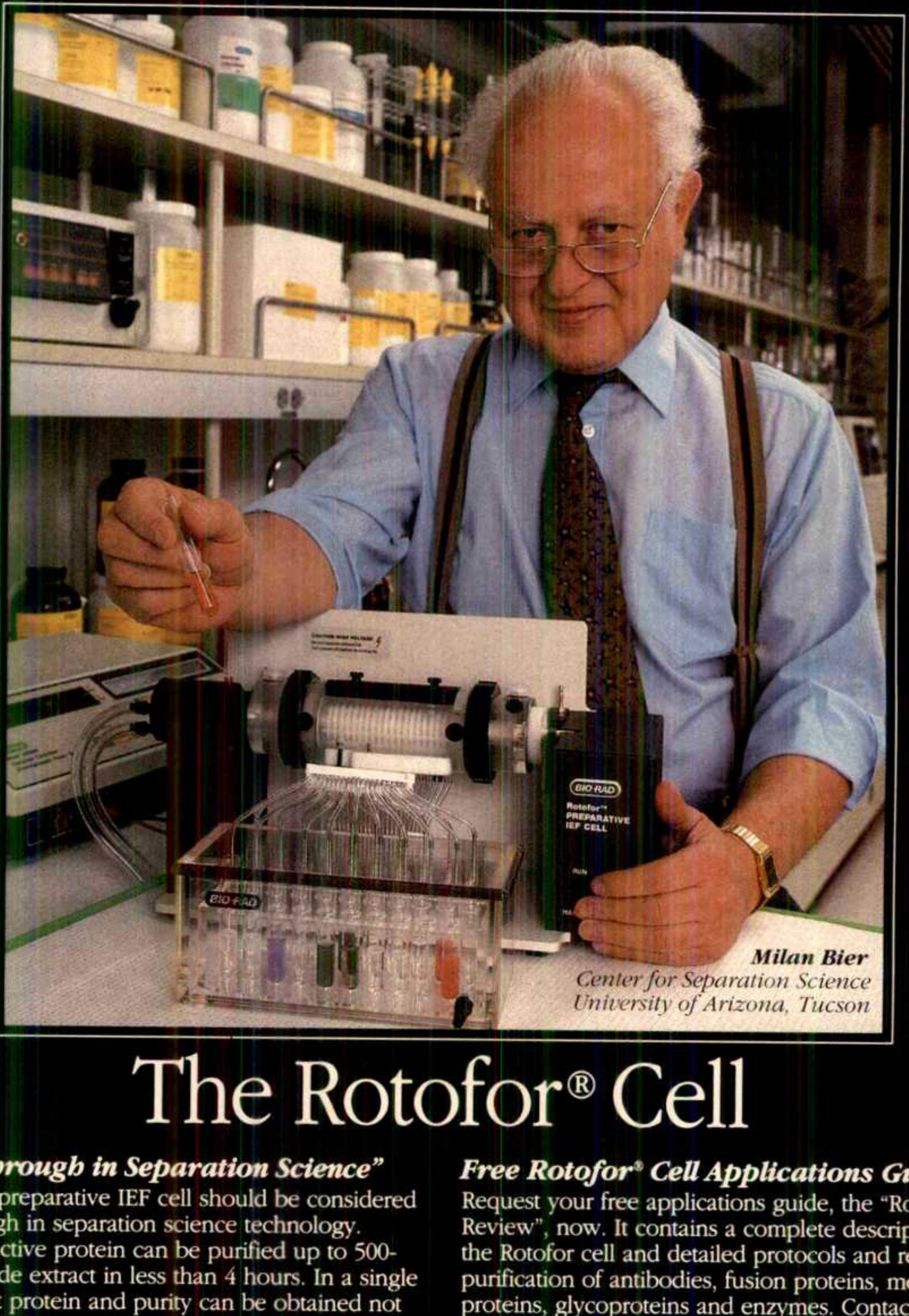

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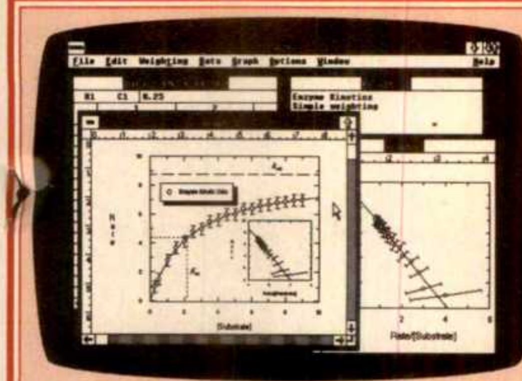

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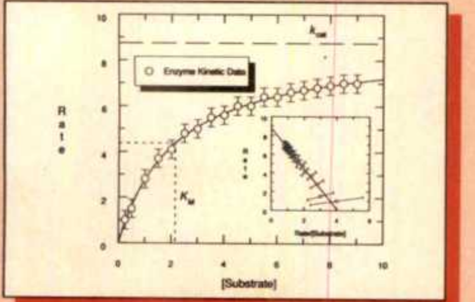

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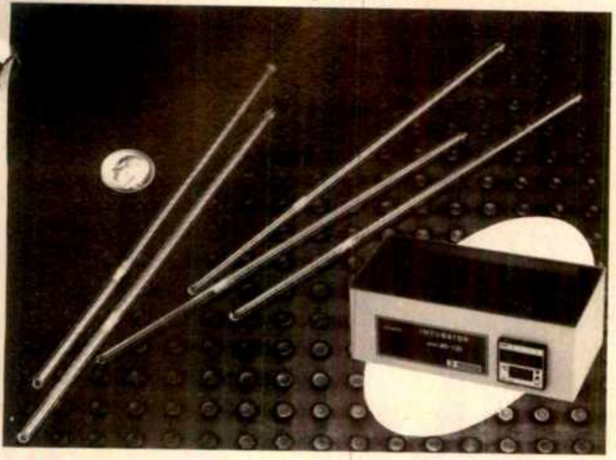

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Understanding gel electrophoresis

Pulsed-field electrophoresis, the newest version of a standard technique, has become widely used for the sieving of large DNA molecules long in advance of an understanding of exactly how it yields useful results.

ELECTROPHORESIS may be one of the most widely used techniques of molecular biology, but its practice is still a craft. Some researchers, or their technicians, have acquired reputations for particular skill in the preparation of gels, or in the interpretation of the records won this way. And while the principles of gel electrophoresis are simple enough — the rate at which protein or DNA molecules are dragged through the aqueous interstitial network running through an inert polymer skeleton by an electric field depends on the length of the molecules, often a good proxy for mass — the limitations of the technique have usually been discovered the hard way (by experiment) before they have been accounted for by calculation.

That is why major innovations in gel electrophoresis have usually also arisen empirically. That is notably the case with the latest development, that of pulsed-field electrophoresis, in which the direction of the forcing electric field alternates at regular intervals between a forward direction and one that may be diametrically opposite or even include a substantial orthogonal component. (The technique, due to David C. Schwartz and Charles R. Cantor, is described in *Cell* **37**, 67; 1984.) The technique has made it possible to separate larger molecules than are accessible when the electric field has a single direction — up to roughly 2 million nucleotide bases, compared with the limit of about 30,000 bases for the conventional technique. But why?

Hand-waving explanations are plentiful, but throw little light on how to optimize the new technique for the separation of molecules of particular size and conformation. Because the applied electric field (usually no greater than a few volts per centimetre) is invariably in the plane of the thin gel, the problem is that of the migration of long molecules in two dimensions. Most of the medium is aqueous, with the polymer skeleton (polyacrylamide or agarose) represented as a random scattering of physical obstacles which long molecules cannot penetrate but on which they can become entangled. Because the medium is aqueous, DNA molecules therein are uniformly charged along their length. In an electric field, they may be expected to drift in that direction. But at what rate?

This is where the fun begins. Suppose that, against all the odds, a DNA molecule is already stretched in the direction of the electric field, which in the simplest case is constant. The molecule has a leading end (the 'head') and a trailing end (the 'tail'). The head must find a path through the solid obstacles randomly presented by the gel, and the remainder of the molecule must, snakelike, follow (whence the description of the movement as 'reptation').

The simplest view of what happens is to suppose that the molecule moves within a tube, more or less in the direction of the field — that defined by the path sought out by the head of the molecule. But there is an obvious difficulty. The unthinking head, subject to unavoidable thermal fluctuations, will hunt in one direction and another for a path through the obstacles in its way, but the path for the remainder of the molecule is already defined, so that the speed at which the tail end sections move behind the head will be determined only by the time taken for thermal fluctuations, as biased by the electric field, to carry them along the tube.

The mobility of the molecule as a whole will be determined by the likelihood that its successive parts will move in the direction of the electrical bias. This is essentially a random walking problem, so that the mobility is proportional to the square root of the length (or mass) of the molecule. So the resolution of electrophoresis should be inversely proportional to the length of the molecules whose separation is attempted.

Sadly, there are complications. For one, even on the assumption that the migration of the molecule represents motion along some notional tube, its trailing sections will travel faster than the head, so that a molecule that began as a stretched-out string will become a more tightly knit conglomerate. The longer the molecule or the stronger the electric field, the more quickly this will happen, when the mobility will be determined by the resistance to the passage of the tangle, whose dimensions will vary only slowly (say, as the one-third power) with length. The mobility of large molecules will be anomalously greater than expected, and sufficiently large molecules will be indistinguishable from each other by their mobility.

Simple (indeed, over-simple) as it is, this picture gives a hint of how pulsed-field electrophoresis may help with the resolution of larger molecules. If the field is reversed, the trailing tangle will be partly unravelled, restoring molecular mobility at least for a time. Obviously, the reversed field must not last for longer than the direct field, or there would be no resolution of even short molecules unaffected by the tangling of trailing molecular sections.

Unfortunately, this picture is a drastic simplification, which takes no account of how molecules become entangled with the gel lattice. So, for the past five years, there has been a steady sprinkling of attempts to model the movement of polymer chains through gel lattices, supposed to consist of regular arrays of obstacles. The general idea is to represent the molecule by a notional string of beads, each carrying an electric charge, which may be connected inelastically or (more cleverly) by elastic springs. The problem is to estimate the rate at which such a structure moves through the lattice of obstructions.

J. M. Deutsch and T. L. Madden were among the first to show that there may be a curious and unexpected oscillation of a normally migrating polymer chain between the extended and tangled structures (*J. Chem. Phys.* **90**, 2476; 1989). Now, Monica Olvera de la Cruz, Dilip Gersappe and Edward O. Shaffer from Northwestern University, Illinois, have described an even more elaborate calculation (using a Cray machine) that throws a little more light on what happens in pulsed-field electrophoresis (*Phys. Rev. Lett.* **64**, 2324; 1990).

The oscillation between the bunched and extended configurations shows up clearly in calculations of the instantaneous radius of gyration (root mean square radius) of the migrating molecules. The beneficial effects of intervening pulses of electric field in a transverse direction show up both in calculations of molecular mobility and in snapshots of the simulations: a transverse field opens up tangled structures, assisting their migration in the principal direction (which nicely confirms the pictures reported last year by Schwarz and Koval in *Nature* **338**, 520; 1989). It is even possible qualitatively to relate the relative duration of the direct and transverse fields to the length of the large molecules for which the elecrophoretic resolution will be greatest. Yet there is a long way to go before the problem can be said to be neatly tidied up — by which time, no doubt, there will be other innovations to account for.

John Maddox

INTRACELLULAR TRANSPORT

Vesicular consumption

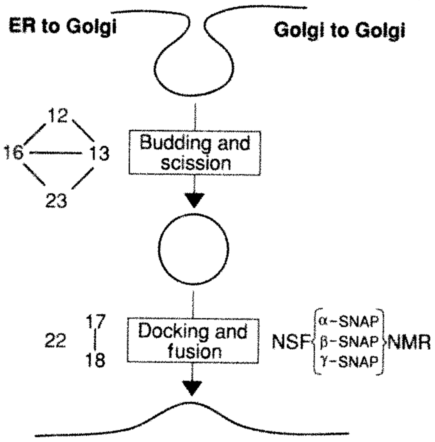
Graham Warren

TRAFFIC of proteins between intracellular membranes is discontinuous, the process being mediated by coated vesicles which bud from one membrane and fuse with the next membrane in the pathway[1]. Budding from the plasma membrane is mediated by adaptors which link clathrin coat subunits to the cytoplasmic tails of receptors to be internalized[2]. Rearrangement of the clathrin lattice causes invagination to form the vesicle, which is released by scission and then uncoated in preparation for fusion with endosomes[3-5]. Fusion is best understood at the level of Golgi transport[6], and although the mechanism is still obscure, two of the proteins involved have been purified. The first, NSF (for *N*-ethyl maleimide-sensitive factor)[7], is involved in fusion after the uncoating of the bound transport vesicle[8]. The second, factor B (ref. 9) (renamed POP (ref. 10), for protein operating in pre-fusion), is involved at a step just before bilayer fusion. Subsequently, NSF was shown to be involved in other vesicle-mediated steps[11,12], implying that it is part of a general fusion machine. Three recent studies — by Kaiser and Schekman[13], Clary and Rothman[14] and Clary *et al.*[15] — together identify another component of this machine using genetic and biochemical approaches.

The genetic experiments involved re-examination of yeast secretory (*sec*) temperature-sensitive mutants that prevent transport from the endoplasmic reticulum (ER) to the Golgi apparatus at the restrictive temperature. By definition, all accumulate ER, but earlier work had shown that some also accumulate small vesicles that seemed to be derived from the ER[16,17]. Using permanganate fixation to highlight these vesicles so that they could be counted, Kaiser and Schekman[13] have now shown that the mutants can be divided into two classes (see figure). The first (*sec12*, *sec13*, *sec16* and *sec23*) cannot produce vesicles at the restrictive temperature; the second (*sec17*, *sec18* and *sec22*) cannot consume them, so thousands accumulate in each cell. This classification predicts that double mutants, constructed from a member from each class, would be unable to produce vesicles — this was observed in all cases.

Lethal phenotype

Other double mutants, constructed from members of the same class, mostly yielded a lethal phenotype, a phenomenon known as synthetic lethality. One possible explanation for this is that although the mutant protein changes to a functional conformation at the permissive temperature, the overall structure still differs from the native protein. This will not matter if the protein with which it normally interacts is native, but if it, too, is mutant, and also differs structurally at the permissive temperature, then the combined differences may clash to give a completely nonfunctional, lethal complex. The absence of lethality does not eliminate the possibility of protein–protein interaction but its presence strongly suggests it. For the *sec* mutants involved in the production of vesicles, five out of the six possible pairwise combinations are lethal. This suggests considerable interaction, which might be expected if the encoded proteins bear any resemblance to the components involved in the budding of endocytic vesicles. By contrast, of the three *sec* mutants involved in vesicle consumption, only one pair is lethal, *sec17* and *sec18*. Because *SEC18* encodes NSF (ref. 18), *SEC17* should encode a protein that interacts with NSF. This protein has now been identified and its interaction with NSF confirmed in an elegant series of experiments[14,15].



The two classes of *SEC* gene involved in ER-to-Golgi transport are shown on the left, the connecting lines showing the lethal pairwise combinations[13]. The proteins involved in fusion of Golgi transport vesicles are shown on the right. *SEC18* is NSF (ref. 18), *SEC17* is α-SNAP (ref. 15). NMR, NSF membrane receptor[19] which has still to be properly characterized.

NSF is a peripheral membrane protein which is isolated in a soluble form[7] and will bind to Golgi membranes only in the presence of a cytosolic fraction termed SNAP (soluble NSF attachment proteins)[19]. By using transport activity as the assay, Clary and Rothman[14] purified to homogeneity three proteins that operate at the same stage of transport as NSF. Clary *et al.*[15] went on to show that these proteins have SNAP activity. The purified SNAPs (α, β and γ) each bind NSF to Golgi membranes and work independently of one another. The amount of SNAP needed to bind NSF to membranes is very similar to the amount needed for vesicle transport, providing the best evidence so far that NSF must be bound to Golgi membranes in order to function. Further experiments showed that SNAP proteins alone would bind NSF and therefore that they link the NSF directly to the Golgi membrane receptor (NMR; see figure).

Yeast cytosol was demonstrated to have SNAP activity enabling *sec* mutants to be screened. The *sec17* mutant was inactive and activity could be restored by the addition of α-SNAP but not β- or γ-SNAP. This result both complements and confirms those obtained by Kaiser and Schekman. It also predicts that the yeast genes encoding β- and γ-SNAP should yield *sec* mutants that accumulate vesicles.

Binding proteins

What then is the role of each of the three SNAPs? They could be viewed as members of a large family of binding proteins, each operating at a different step in the pathways of membrane traffic, binding NSF to target membranes. As the authors of the new papers argue, however, this does not explain the complementation of *sec17* by α-SNAP. The *sec17* mutant interrupts transport from the ER to Golgi, yet α-SNAP was identified by its ability to transport vesicles from *cis* to medial cisternae.

Therefore, α-SNAP operates at more than one step. Instead, as the authors point out, the proteins should be viewed as comprising a particle containing NSF, α-, β- and γ-SNAPs. It is then necessary to explain why any one of the SNAPs alone can substitute for the other two in transport and binding assays. In the binding assays, one can imagine that any one of the SNAPs might suffice to bind NSF to Golgi membranes. In the transport assay, however, all should be needed if the particle is to function. The authors argue that washed Golgi membranes may still contain residual SNAPs in amounts sufficient

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1. Palade, G. *Science* **189**, 347–358 (1975).
2. Pearse, B.M.F. *EMBO J.* **7**, 3331–3336 (1988).
3. Heuser, J. & Evans, L. *J. Cell Biol.* **84**, 560–583 (1980).
4. Smythe, E. *et al. J. Cell Biol.* **108**, 843–853 (1989).
5. Rothman, J.E. & Schmid, S.L. *Cell* **46**, 5–9 (1985).
6. Rothman, J.E. & Orci, L. *FASEB J.* **4**, 1460–1468 (1990).
7. Block, M.R. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 7852–7856 (1988).
8. Malhotra, V. *et al. Cell* **54**, 221–227 (1988).
9. Wattenberg, B.W. & Rothman, J.E. *J. biol. Chem.* **261**, 2208–2213 (1986).
10. Wattenberg, B.W. *et al. J. Cell Biol.* **110**, 947–954 (1990).
11. Beckers, C. J. M. *et al. Nature* **339**, 397–398 (1989).
12. Diaz, R. *et al. Nature* **339**, 398–400 (1989).
13. Kaiser, C.A. & Schekman, R. *Cell* **61**, 723–733 (1990).
14. Clary, D.O. & Rothman, J.E. *J. biol. Chem.* (in the press).
15. Clary, D.O., Griff, I.C. & Rothman, J.E. *Cell* **61**, 709–721 (1990).
16. Novick, P. *et al. Cell* **25**, 461–469 (1981).
17. Esmon, B. *et al. Cell* **25**, 451–460 (1981).
18. Wilson, D.W. *et al. Nature* **339**, 355–360 (1989).
19. Weidman, P. J. *et al. J. Cell Biol.* **108**, 1589–1596 (1989).
20. Goud, B. *et al. Cell* **53**, 753–768 (1988).
21. Baker, D. *et al. Cell* **54**, 335–344 (1988).
22. Ruohola, H. *et al. J. Cell Biol.* **107**, 1465–1476 (1988).

to complement the exogenously added SNAP. But if each purified SNAP can be complemented, all must be present on the membrane, so why would any exogenous SNAPs be needed? Further work is required both to resolve this question and to define more precisely the involvement of each of the components in this complex fusion machine.

The study of membrane traffic has in the past been dominated by morphological and biochemical techniques[1,6]. The *sec* mutants were first described ten years ago[16], but, with one notable exception[20], they have not in themselves given insight into the mechanism of action of the molecules they encode. One reason has been the lack of cell-free assays that mimic steps on the secretory pathway, although considerable progress has been made in the past few years[21,22]. Another and more serious reason is the absence of good morphological techniques for studying the pathways of membrane traffic in yeast. Here is an area where microscopists willing to make the effort will reap the reward of raising the morphological quality to the level long enjoyed by animal cell microscopists. Kaiser and Schekman show what can be done when one combines yeast genetics and morphology, but the fact that they had to use such a drastic staining technique, which destroyed much of the cell's morphology and reduced the vesicles they were measuring to amorphous, metallic blobs, serves to emphasize the effort that still needs to be applied in this area. In the absence of such effort, morphological approaches to this problem will continue to depend upon hybrid systems in which animal membranes are complemented by yeast components. ☐

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EARTHQUAKES

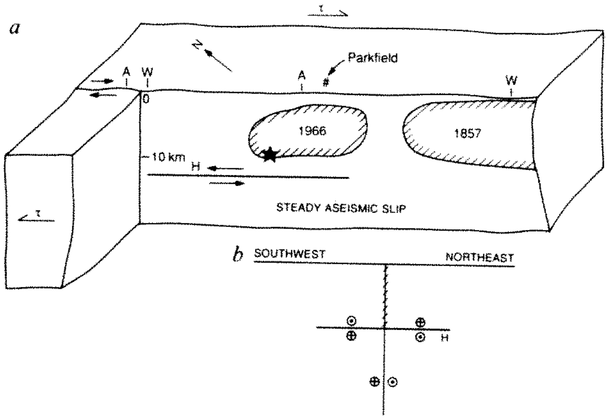
Parkfield slowing down

William D. Stuart

EARTHQUAKE precursors are so elusive that their detection requires dense arrays of sensitive instruments close to the area of earthquake rupture. But to select an array site one first has to predict coarsely by other means where the earthquake will be. For the San Andreas fault near Parkfield, California, a coarse prediction has been provided by the roughly periodic occurrence of similar moderate earthquakes (magnitude 5.5–6) in 1966, 1934, 1922, 1901, 1881 and 1857 (ref. 1). From these dates alone the expected time of the next event was 1988 with an uncertainty of ± 7 years. Using data from a dense seismometer array operated by the US Geological Survey in anticipation of the next earthquake, Wyss *et al.*[2] on page 426 of this issue report a rate decrease of small-magnitude seismicity starting in 1986. They interpret this quiescence as a precursor and estimate that the next moderate earthquake will occur in March 1991 ± 1 year. Thus, a long-term prediction has enabled an intermediate-term one to be made.

Wyss *et al.* estimate the time of the impending earthquake by comparison with six previous periods of quiescence that preceded a mainshock. The case histories used were for earthquakes of magnitudes 3.6–8 in California, Hawaii and Alaska. Curiously, the duration of the period of quiescence for these cases varies from 1.5 to 3.8 years, suggesting a weak dependence on the earthquake magnitude. But the durations and earthquake magnitude are positively correlated.

Having so few case histories and lacking a physical theory, Wyss *et al.* acknowledge that the uncertainty of ± 1 yr is poorly constrained. If one accepts the case histories, the four-year lapse since the onset of quiescence is actually more consistent with a magnitude 7 earthquake than a magnitude 5.5–6 earthquake. The San Andreas fault just southeast of Parkfield is a likely place for a magnitude 7 event[3]. That fault section has been locked since the magnitude 8.3 Fort Tejon earthquake in 1857, and the amount of slip now available for release during an earthquake is 4–5 m.



a, Cutaway view of San Andreas fault at Parkfield. Regional stress tending to deform the Parkfield area is shown by τ. Paired arrows show sense of fault slip. Hatching outlines currently locked fault patches. '1966' marks the patch expected to fail again soon in a moderate earthquake (star is the focus of the 1966 earthquake); '1857' marks the tip of the patch that failed to make the $M = 8.3$ Fort Tejon earthquake. A and W mark the edges of the crustal volumes used by Aviles and Valdes[4] and Wyss *et al.*[2] to detect seismic quiescence. H marks the position of the conjectured horizontal fault responsible for both quiescence and the rate decrease of geodetic lines. *b*, End view of fault showing relative displacements for fault slip. The dot and plus sign indicate motion out of and into paper, respectively.

The quiescence in the Parkfield earthquake catalogue was also noticed by Aviles and Valdes[4]. They found that the occurrence rate of earthquakes of magnitude greater than 2 decreased over a crustal volume starting about half way into the rupture area of the 1966 earthquake and extending 30 km northwest along the fault (see part *a* of figure). The crustal volume studied by Wyss *et al.*, on the other hand, is 54 km long and essentially encloses both the volume of Aviles and Valdes and the entire 1966 rupture area (since healed). Both groups agree that the strongest quiescence is in the smaller volume, but it is unclear why the strongest quiescence should enclose just the north end of the 1966 rupture and not all of it.

Reasenberg and Matthews[5,6] formulated a precise statistical procedure for a quiescence search in an earthquake catalogue and dispute some of the quiescences claimed by Wyss *et al.*[2]. In particular, they argue that the claimed statistical significance of quiescences is too high, and that when the correct statistic is used some rate changes of seismicity that seem to be visually significant are in fact not. It may be that only certain fault geometries or slip modes have quiescences.

The case for the Parkfield quiescence is considerably strengthened by geodetic distance measurements made by a two-colour laser-ranging instrument. In a companion paper on page 428 of this issue, Wyss *et al.*[7] notice that the rate of shortening of two geodetic survey lines crossing the San Andreas fault decreased by about 20 per cent within a few months of the start of the period of quiescence. So there is good evidence of either some physical change in the fault or an alteration of loading rate. One explanation suggested by Wyss *et al.*[7] is an increase in the fraction of the fault zone that is locked. Overall, the fault would become stronger and consequently deform at a lower rate. A lower deformation rate implies decreased production of background seismicity and slower motion of bench marks at the ends of geodetic lines.

Another model that comes to mind is a simple revision of the generally accepted model for stressing of the fault near Parkfield. In the current model (see figure) the top 10–20 km of the San Andreas fault is locked from the 1966 rupture south. Elsewhere the fault slides aseismically, loading the locked patches towards failure. Moderate earthquakes, such as the 1966 event, occur during failure of the left patch shown. Otherwise, the stress increase on and near locked patches generates background seismicity and deformation of the ground surface.

In the revised model, seismicity and geodetic-rate decreases are caused by slip on a sub-horizontal fault (H in figure) beneath the locked patches. The same deep slip on the San Andreas fault that loads the locked patches also loads a horizontal plane at H, and if the plane's yield stress is a little less than that of the overhead patch, then the horizontal fault will slip first. Slip on the horizontal fault will be late in the seismic cycle and will decrease the loading rate on the vertical fault and adjacent crust. The postulated fault may be part of the sub-horizontal detachment fault associated with the magnitude 6.7 Coalinga earthquake in 1983, 40 km northeast of Parkfield. In short, seismicity and crustal motion slow down late in the earthquake cycle because the detachment fault, acting as a buffer, comes to life.

The reason for seeking precursory anomalies is, of course, to make short- or intermediate-term earthquake predictions. The advantage of such predictions is that they may be more precise than long-term ones. Long-term predictions (more than a few years), such as those derived from earthquake-recurrence intervals, usually have uncertainties of many years. This is true even when long-term predictions are refined using conditional probabilities to allow for the time elapsed with no earthquakes[3]. As Savage[8] puts it, the probabilities are uninformative because the acceptable range of probability values is so great.

The empirical approach of Wyss *et al.*[2,7] is one way to make an intermediate-term prediction from observations. But their work also places within reach a mechanical model connecting background seismicity, crustal deformation and a forthcoming mainshock. A detailed and accurate model might therefore be adapted to forecast earthquakes just as atmospheric models are used to forecast the weather. □

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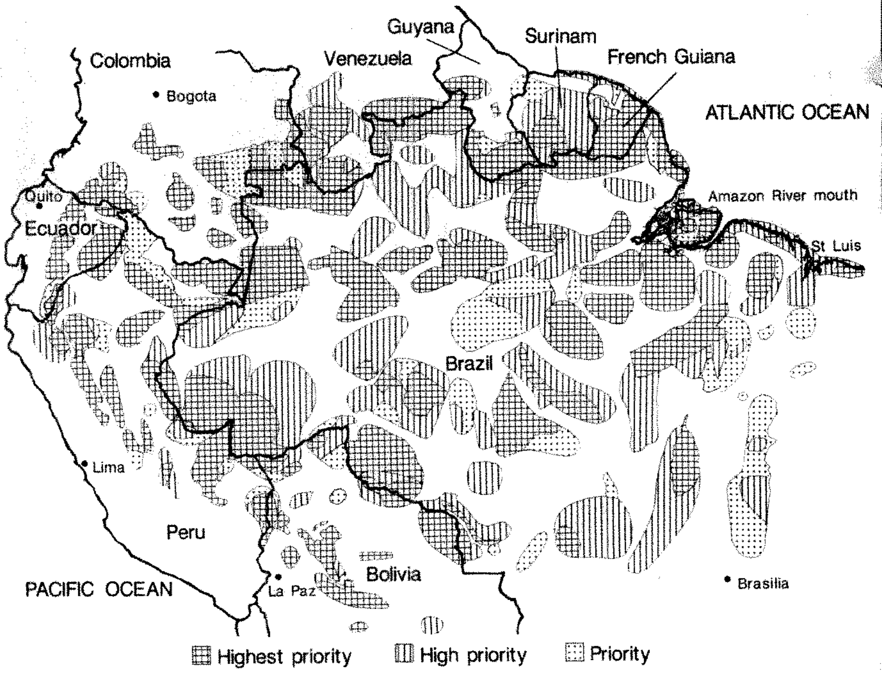
1. Bakun, W.H. & Lindh, A.G. *Science* **229**, 619–624 (1985).
2. Wyss, M., Bodin, P. & Habermann, R.E. *Nature* **345**, 426–428 (1990).
3. *US Geol. Surv. Open-File Rep.* 88–398, (USGS, 1988).
4. Aviles, C.A. & Valdes, C.M. *Eos* **70**, 1228 (1989).
5. Reasenberg, P.A. & Matthews, M.V. *Pure appl. Geophys.* **126**, 373–406 (1988).
6. Matthews, M.V. & Reasenberg, P.A. *Pure appl. Geophys.* **126**, 357–372 (1988).
7. Wyss, M., Slater, L. & Burford, R.O. *Nature* **345**, 428–431 (1990).
8. Savage, J.C. *Seismol. Res. Lett.* **61**, 43 (1990).

Consensus for conservation



THE map reproduced here, the fruit of a recent interdisciplinary meeting*, identifies the areas of biological priority for conservation in Amazonia, graded into three levels. The total area shaded covers about 60 per cent of the Amazon basin and the areas with square hatching are of greatest biological importance. The map represents the consensus of some 100 biologists, physical scientists and conservation planners — it will be an essential tool in planning the location of biological reserves, national parks, extractivist reserves and other conservation areas in Amazonia.

Scientists at the meeting began by working in small specialist groups: plant systematics, plant ecology, mammals, ornithology, herpetology, icthyology, entomology, geomorphology and climate, and units of conservation. The seven biological groups each produced thematic maps to justify their selection of priority areas. This was followed by separate meetings of botanists and zoologists to pool their data and consolidate their chosen areas on maps for botany and for zoology; as it turned out the areas selected by the different groups broadly corresponded. At the same time, the geomorphologists and climatologists produced a map identifying the most fragile soils and the ecosystems requiring the greatest protection. The conservation unit's group discussed policy and other factors bearing on the preservation, conservation and management of priority areas.

The last step was the fusion of the 104 areas of biological importance for botany with the areas selected by zoologists. The final map shows 94 priority areas, evaluated on a five-point scale (eventually reduced to three). Areas with the maximum overlap between disciplines were given the highest priority.

Much on-site work remains to be done by conservation organizations of the nine countries concerned, to select the precise areas suitable for different categories of reserves, managed forests and regions for the indigenous population. The shaded parts of the map include some deforested and inhabited areas and site visits are necessary to locate suitable reserves. But the biologists have provided their data, and now it is the turn of conservation planners to choose appropriate reserves, politicians to make them a reality and international assistance organizations to help finance them.

What of the white areas? Are these the regions in which deforestation should be allowed to proceed? They are indeed probably of less importance in terms of species diversity, but the state of inventory of the Amazon region is still so poor that it would be unwise to advocate development projects in these spaces that, in many cases, represent some of the least explored parts of Amazonia. These are areas that need to be chosen for further inventory, rather than for deforestation. Ghillean T. Prance

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**Workshop 90*, Manaus, Brazil, 10–20 January 1990.

ARCHAEOLOGY

Agricultural renascence in the high Andes

Warwick Bray

ONE of the saddest sights in Peru and Bolivia is the vast extent of farmland that was intensively cultivated in prehispanic times and is now all but abandoned. Between 50 per cent and 75 per cent of Inca terraces are no longer in use, and in some of the coastal valleys there was up to 40 per cent more irrigated land in pre-Conquest times than there is today (Clark Erickson, manuscript in preparation). Moreover, entire technologies have been forgotten: the *qochas* (circular field basins) of the high Andes, the sunken gardens in areas of high ground-water along the coast, and the systems of raised fields in waterlogged areas at all altitudes. The remains of these indigenous technologies are now being rediscovered by archaeologists and geographers, and the implications have forced government agronomists and foreign-aid agencies to take notice.

The most sophisticated of the current projects are located in the basin of Lake Titicaca, on the Peru–Bolivia border at about 3,800 m above sea level. Aerial photographs revealed more than 83,000 hectares of eroded and abandoned fields on the lakeshore plain in terrain nowadays used only as low-grade pasture. The zone adjacent to the lake is perennially marshy and is subject to flooding in the wet season. These problems are compounded by efflorescence of salts as a result of evapotranspiration, and by the risk of sudden frosts that can wipe out an entire harvest.

Investigations began in 1981–86 when Erickson excavated a group of raised fields near Huatta, on the Peruvian side of the lake[1,2]. He showed that between 1000 BC and AD 400 the inhabitants of the region dug a series of parallel canals, and piled the earth between them to form long, low mounds about 1 m high, 4–10 m wide and 10–100 m long. Pollen from the field soils indicates that potatoes and quinoa (a high-altitude grain rich in protein) were the main crops. The canals provided moisture during periods of drought, and were also a source of green mulch and organic muds to fertilize the fields, thus eliminating the need for fallowing. The raised planting surface helped to prevent waterlogging of roots, and the canals served as traps for sediment (which could be scooped out and recycled) and also as heat sinks, warming up during the days and slowly releasing heat at night. Measurements show that the night temperature on raised fields in conditions of light frost was about 2 °C higher than that of control plots, and that the frost was several hours shorter in duration. The effect was not merely to reduce frost damage, but also to extend the growing season.

In the Huatta region, construction of raised fields ceased by about AD 400, a date that coincides with the growth of the city of Tiwanaku in the southern (Bolivian) part of the basin. In the rural hinterland around Tiwanaku, Kolata and Ortloff are studying the agricultural landscape that provided food for one of the main prehistoric civilizations of the Andes. They show that the landscape of the Tiwanaku period (about AD 400–1100) was almost entirely man-made, with terraces on the hill slopes and systems of raised fields, interspersed with farms and townships, on the lakeshore plain[3,4]. Using mathematical models to study water flow and the effects of fluctuating water levels on drainage, salinization and heat-storage capacity, they demonstrate that the landscape functioned as an integrated system. Wet-season rainfall in the hills was collected in modified natural gullies and transferred by means of aqueducts across the plain and into the lake. Canalizing runoff prevented deposition of unwanted sediment and, more importantly, controlled the water table by reducing infiltration of surface water into the groundwater reservoir.

Hutchison Library

The shores of Lake Titicaca, in prehispanic times a site of intensive agriculture.

But could this kind of system work today? The answer is 'yes'. On experimental raised fields, sustainable potato yields are twice those of the control plots, and often much more. In years when frost destroyed harvests on conventional plots, losses of potatoes, barley, quinoa and vegetables on the raised fields were minimal.

Above all, as Erickson and his colleagues emphasize[1,2], raised-field technology requires no imported seed, chemical fertilizers or expensive machinery, is based on traditional crops and tools, and does not disrupt existing work patterns and social norms. In contrast, other competing schemes have been capital-intensive or designed to produce cash crops from which most small farmers have not benefited. By starting at the bottom, with the villagers themselves (rather than imposing from the top), by setting a practical example, and by initiating a training programme with simple manuals in Spanish and Quechua, the Huatta team has persuaded several local communities to build raised fields for themselves. Similar experiments have begun on a small scale in the very different conditions of coastal Ecuador[5], and Colombia (with more than 500,000 hectares of abandoned raised fields in the Caribbean lowlands[6]) is also showing interest. □

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1. Erickson, C. L. & Candler, K. L. in *Fragile Lands of Latin America: Strategies for Sustainable Development* (ed. Browder, J. O.) 230–249 (Westview Press, Boulder, 1989).
2. Erickson, C. L. *Expedition* **30**(3), 8–16 (1990).
3. Kolata, A. L. & Ortloff, C. *J. archaeol. Sci.* **16**, 223–263 (1989).
4. Ortloff, C. R. & Kolata, A. L. *J. archaeol. Sci.* **16**, 513–535 (1989).
5. Muse, M. & Quintero, F. in *Pre-Hispanic Agricultural Fields in the Andean Region* (eds Denevan, W. M., Mathewson, K. & Knapp, G.) 249–266 (British Archaeological Reports, International Series 359, Oxford, 1987).
6. Plazas, C., Falchetti, A. M., van der Hammen, T. & Botero, P. *Boletin del Museo del Oro* 55–88 (1988).

MICROSCOPY

Electrons give a broader view

Archie Howie

THE dazzling success of optical holography cannot blind us to the fact that Gabor's proposal[1] in 1948 for atomic resolution by electron holography has so far been realized only partially, despite substantial advances in electron microscopy[2,3]. Some new proposals[4-6] for lensless holographic imaging of adsorbed surface structures using low-energy electrons may, however, have brought the prospect distinctly closer.

Electron diffraction patterns, Fresnel fringes and other wave-interference effects are easily demonstrated. But to record a hologram based on electron wavelengths, considerable magnification is needed, either through geometrical projection or with electron lenses still restricted by spherical aberration to an angular aperture of about 1°. Stray a.c. magnetic fields are often critical because they destroy interference effects. Moreover, the strong electron–atom interaction demands that a thin sample be used to minimize multiple elastic scattering and avoid the equivalent of the optical hologram of a chess set that is so highly reflective that each piece is illuminated mainly by light coming from the others by a variety of interference paths rather than directly from the source. Inelastic scattering is also likely, destroying any interference effects except in situations where the reference and scattered beams are involved in the same inelastic scattering event.

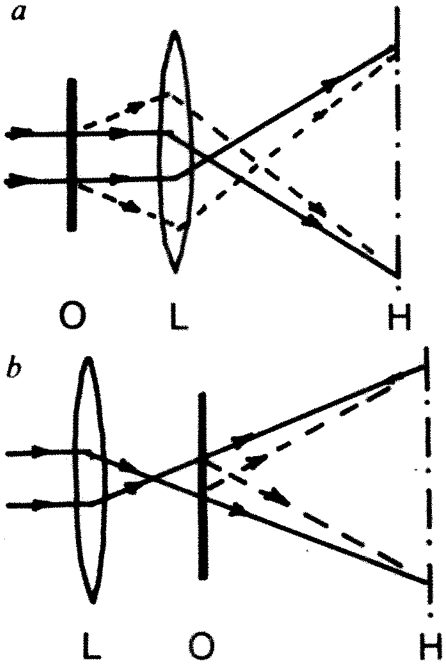

FIG. 1 Schematic illustrations of the use of a lens L to produce a transmission hologram H of a transparent object O by interference of a reference wave (continuous line) and a scattered wave (broken line). The standard arrangement for high-resolution electron microscopy is shown in *a*; Gabor's projection scheme[1] is shown in *b*.

High-resolution transmission electron microscopy overcomes these problems by using as a reference wave that part of the incident beam that is transmitted without deflection through the specimen. The objective lens deflects the waves scattered by the specimen back to the axis where they interfere with the reference wave to form an on-axis, image plane hologram as shown in Fig. 1*a*. This hologram can be recorded on a photographic plate after further magnification. Thin samples behave like 'phase objects' (perfectly transparent but inducing a phase change) and a combination of defocus and spherical aberration is used to obtain amplitude contrast in the hologram, typically restricted to a range of object spacings between 2 and 0.15 nm. Critical extraneous a.c. magnetic fields are in the milligauss region. These image holograms can be viewed directly as electron micrographs without any reconstruction procedure and, because of the restricted lens aperture, look like two-dimensional projections of the three-dimensional object structure. The temptation to interpret them literally — and sometimes erroneously, because of complications from instrumental aberrations, multiple scattering or projection effects — has generally proved irresistible.

An electron–optical biprism incorporated in the microscope column allows more ambitious off-axis holographic imaging of phase-object-contrast detail from magnetic structures[2], p–n junctions[7] and surface steps[8] and may also enable the high-resolution limit to be pushed down to scales of 0.1 nm and below[9]. Gabor's geometrical magnification scheme[1] has been re-examined[9] using a high-resolution scanning transmission microscope to form a focused source close to the object, as shown in Fig. 1*b*. In principle, the method will work if the instrumental aberrations are sufficiently well known to specify accurately the complex amplitude distribution in the source, but additional difficulties arise because of mechanical vibration and specimen drift. The restricted angular aperture of electron lenses, whether before or after the object, is also a continuing obstacle to genuine three-dimensional imaging at high resolution.

The proposal[4] for surface holography elegantly achieves a coherent point source fixed to the sample by causing one of the surface atoms of the sample to emit either low-energy photoelectrons or (following either photon or electron excitation) Auger electrons. As shown in Fig. 2, a hologram is then formed by simple geometrical projection of these electrons onto a hemispherical screen or energy-selective angular detector, where a reference wave coming directly from the source atom can interfere with waves that arrive after scattering on surrounding atoms. The source atoms can be surface adsorbate atoms, which need not be arranged periodically. Their individual contributions to the hologram intensity will superimpose provided that they each sit on a site with the surrounding atoms in the same angular positions. Reconstruction would involve illuminating the hologram with a coherent converging spherical wave, but this optical problem can be solved numerically by Kirchoff's method. In a numerical simulation[5] of this procedure using theoretical data, a three-dimensional adsorbate atom structure has been successfully recovered. A further, very recent extension of the idea, backed up once again by numerical simulations, is the analysis of diffuse low-energy electron diffraction (DLEED) intensity distributions[6]. The DLEED method is also applied to adsorbate atoms present at fairly low concentration on random surface sites of an underlying crystal. Hitherto, structural information about the adsorbate atom surroundings has been available only by matching the diffuse background intensity in the diffraction pattern to the results of sophisticated computations of multiple scattering in a whole series of candidate structures.

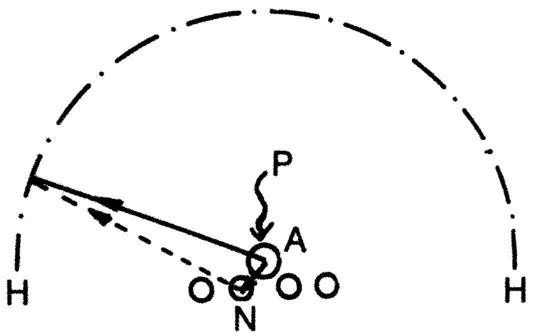

FIG. 2 Schematic illustration of the use of a source atom A (stimulated by a photon or high-energy electron P) to emit a spherical reference wave (continuous lines) interfering with weaker waves (broken line) scattered by neighbouring atoms N. A hologram H is thus formed on a remote spherical screen.

If these numerical simulations are valid, holographic reconstruction eliminates the need for such elaborate computations and offers a comparatively simple and direct route to surface-structure determination. Further theoretical analysis would be useful to justify, for instance, the claim that multiple scattering effects do not disrupt the reconstruction and to clarify the application of Kirchoff theory to a region that is not completely enclosed by a boundary. More seriously, of course, we await a rigorous experimental test. The technique should be quite robust to the influence of vibration and stray fields, but the effects of multiple scattering and inelastic or phonon scattering are less clear. Suitable experimental data is readily to hand, for example the striking so-called ADAM (angular-distribution Auger microscopy) images[10] which have been somewhat naively interpreted as silhouettes of surface atoms, with the remarkable assertion that diffraction effects are not relevant. It may be fair to assume, as is apparently done here, that reconstruction with a converging spherical wave is little different from reconstruction with a wave expanding from a small source at the centre of the hemispherical hologram. 'A ball bearing model of the atomic positions' is however a most unconvincing answer to the interesting question of what object characteristic is recovered when an electron hologram is illuminated with light. The high-resolution microscopists have already found that a naive interpretation of electron holograms could be erroneous. We must hope that the exciting extension of holography to low-energy electron diffraction does not carry with it the same danger. □

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1. Gabor, D. *Nature* **161**, 777–778 (1948).
2. Tonomura, A. *Rev. mod. Phys.* **59**, 639–669 (1987).
3. Lichte, H. *Ultramicroscopy* **20**, 293–304 (1986).
4. Szöke, A. in *Short Wavelength Coherent Radiation: Generation and Applications* (eds Attwood, D.T. & Boker, J.) 361–367 (Am. Inst. of Physics, New York, 1986).
5. Barton, J.J. *Phys. Rev. Lett.* **61**, 1365–1359 (1988).
6. Saldin, D.K. & de Andres, P.L. *Phys. Rev. Lett.* **64**, 1270–1273 (1990).
7. Frabboni, S., Matteucci, G. & Pozzi, G. *Ultramicroscopy* **23**, 29–38 (1987).
8. Banzhof, H., Hermann, K.H. & Lichte, H. in *EUREM 88* (eds Goodhew, P.J. & Dickinson, H.G.) **1**, 263–264 (Inst. of Physics, Bristol, 1988).
9. Lin, J.M. & Cowley, J.M. *Ultramicroscopy* **19**, 179–190 (1986).
10. Frank, D.C., Batina, N., Golden, T., Lu, F. & Hubbard, A.T. *Science* **247**, 182–188 (1990).

GEOCHRONOLOGY

Timescales and telltale corals

Minze Stuiver

WHEN investigating past events, the radiocarbon (^{14}C) dating method is used widely and is usually considered the most reliable for determining ages over the past 50,000 years. Because of its heavy use it is important to identify its imperfections. Bard *et al.* on page 405 of this issue[1] compare the ^{14}C and ^{230}Th/^{234}U ages of Barbados corals and provide a much-needed calibration of the radiocarbon timescale beyond the range of previous calibrations.

The past decade has seen a shift from radioactive decay counting to direct determination by mass spectrometry of minute isotope amounts. Accelerator mass spectrometry now delivers ^{14}C ages with good precision for milligram-sized C samples, and Bard *et al.*'s refinement of standard mass spectrometric techniques gives coral ^{230}Th and ^{234}U determinations an order of magnitude more precise than those obtained by decay counting. With such improved technology, the assumptions underlying each dating method become increasingly important.

The incorporation of uranium in coral as it forms, and the subsequent radioactive decay of ^{238}U and ^{234}U to ^{230}Th allows uranium–thorium ages to be determined. The ^{230}Th/^{234}U and ^{238}U/^{234}U ratios have to be measured, with the initial amount of ^{230}Th calculated from the measured abundance of ^{232}Th. The corals are assumed to represent a closed system for U and Th exchange. This assumption is crucial as U and Th are notoriously mobile in sediments. The absence of variation in the calculated initial ^{238}U/^{234}U ratios of the Barbados corals agrees with these closed-system assumptions, and so Bard *et al.* conclude that the U/Th systematics could not have been strongly altered after deposition. Also consistent with a closed system is the similarity of the ^{230}Th/^{234}U to the calibrated ^{14}C ages of the past 10,000 years. The absence of any indications of exchange is, however, not necessarily proof of a closed system but it seems that post-depositional alteration is unlikely.

Determining a conventional ^{14}C age in the laboratory involves measuring the ^{14}C activity remaining in the sample, normalizing this activity to a fixed δ^{13}C value to eliminate the influence of isotope fractionation, and calculating the ratio R of normalized sample activity to the activity of an NBS standard that represents atmospheric ^{14}C activity of the late nineteenth century. The ratio R is then converted to a conventional ^{14}C age using the radioactive decay law. The crux of the matter is, of course, that the atmospheric ^{14}C activity of the late nineteenth century does not necessarily represent the initial ^{14}C activity of samples grown at different times and in diverse reservoirs differing in ^{14}C activity from that of the atmosphere. For the latter effect an estimate of the ^{14}C age difference with the atmosphere (reservoir correction) has to be made; whereas for the former, information from other dating methods is needed.

The 'fine tuning' or calibration of ^{14}C ages has, so far, been achieved using wood dated by dendrochronology (tree-ring counting). Existing wood chronologies are derived from environments as diverse as the slopes of the White Mountains, Nevada (bristlecone pine), the peatbogs of Ireland (oak) and the alluvial sands of the major rivers of Germany (oak). The longest continuous chronology goes back to 7938 BC (ref. 2).

High-precision radiocarbon dating of wood from these chronologies allows radiocarbon ages to be plotted against AD/BC calibrated (cal) ages. These plots[3] are used to convert the radiocarbon age to the AD/BC timescale. Although computerized versions of the calibration process are available[3] the process is not ideal because in many instances several solutions are possible for a single ^{14}C age (maximum uncertainty about 200 cal years)[4].

The ^{14}C age anomalies encountered for the oldest wood samples are fairly large; that is, wood of nearly 10,000 cal yr BP (before present, with 0 BP = AD 1950) has a conventional radiocarbon age of only about 9,000 ^{14}C years. Part of this discrepancy arises from a known 3 per cent shortening of the ^{14}C timescale because the half-life used, agreed upon internationally 40 years ago, is 3 per cent too small, but most of it is caused by an increase in atmospheric ^{14}C activity over time. Do these age anomalies increase further back in time? The ^{230}Th/^{234}U ages of the Barbados corals show unequivocally that before 9,000 yr BP, the ^{14}C ages are systematically younger than the U–Th ages, with differences of about 3,800 years at 20,000 yr BP.

Although the measuring errors in ^{230}Th/^{234}U and ^{14}C coral dating are of comparable magnitude, they differ in their areas of applicability because corals are found only in a portion of the marine domain whereas ^{14}C samples are encountered in all carbon reservoirs. To apply the ^{14}C age correction found for corals to the ^{14}C ages of samples in equilibrium with the atmosphere requires that the time history of the offset ^{14}C ages of the atmospheric and oceanic mixed-layer reservoir be known. This offset, about 400 ^{14}C years for the present-day anthropogenically undisturbed mixed layer of the global ocean, has fluctuated during the Holocene by a few hundred years[5]. The change in offset may have been several hundred years larger before 12,000 cal yr BP because of major changes in ocean deep-water formation related to glacial–interglacial climate change. The coral ^{230}Th/^{234}U results, together with an estimate of the radiocarbon offset between the reservoirs, provides important information on long-term trends in ▸

1. Bard, E. *et al. Nature* **345**, 405–410 (1990).
2. Becker, B. & Kromer, B. *Abst. Int. Symp. Archaeometry Heidelberg*, Weber D-6900 Heidelberg, 192 (1990).
3. Stuiver, M. & Kra, R. (eds) *Radiocarbon* **28**, 805–1030 (1986).
4. Stuiver, M. in *Climate and Geo-Sciences* (eds Berger, A., Schneider, S. & Duplessey, J.) Cl. NATO ASI Math. and Phys. Sci. **285**, 39–45 (1989).
5. Stuiver, M., Pearson, G.W. & Braziunas, T.F. *Radiocarbon* **28**, 980–1021 (1986).

atmospheric ^{14}C. However, our insufficient knowledge of palaeo-ocean circulation precludes detailed calculations of the variations of the offset with time and thus the fine detail observed in the atmospheric tree-ring ^{14}C record and the associated calibration of the radiocarbon timescale cannot be matched.

Variations in atmospheric ^{14}C are caused by changes in production rate associated with solar wind modulation, change in the geomagnetic dipole intensity and redistribution of ^{14}C between the carbon reservoirs induced by climate change. Bard *et al.* argue that the changes in atmospheric ^{14}C of up to 40 per cent which they derived from the coral data are so large that a change in geomagnetic intensity seems to be the only reasonable explanation.

The adjusted ^{14}C timescale shifts the records of global glacial climate, currently set at 18,000 cal yr, back to about 21,000–22,000 cal yr. The putative glacial maximum at 18,000 cal yr is already open to dispute; a move of this sort should stimulate further discussion of the way that the Milankovitch Earth orbital cycles drive climate variations. ☐

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CRYSTALLOGRAPHY

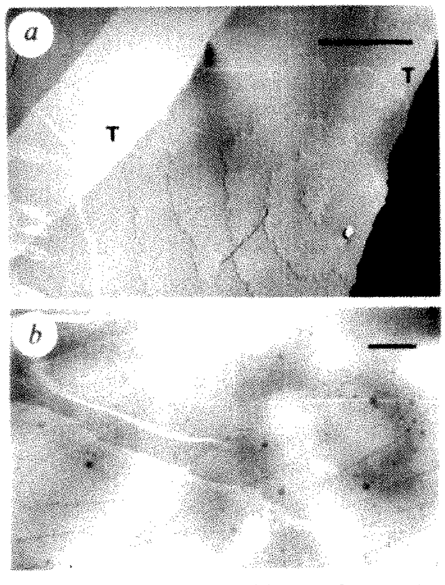
Steps in a growth business

David Dover

THOSE investigating protein structure by X-ray crystallography depend on a reliable supply of sufficiently large, well-ordered crystals on which to work. Improvement of crystallization techniques has been the Cinderella of the business, but has been receiving more attention recently — perhaps because an increasing number of structural studies have stalled owing to crystallization problems. Of the new approaches, the glamour goes to microgravity (in particular) and to robotics, both of which are in the tradition of informed trial-and-error that has been characteristic of the field since its inception[1]. More analytical approaches have been tried in a number of laboratories without success. But now that Durbin and Feher[2] have shown that electron microscopy can reveal much of what is happening, more optimism in the crystal nurseries can be expected.

The advantage of microgravity (growing crystals in satellites in Earth orbit) is that there are no sedimentation or convection currents. If the geometry is chosen correctly, boundaries other than the water–vapour interface can be eliminated too, and the ideal would be to grow crystals in a protein-solution droplet floating freely in space. Although falling short of that ideal, some of the first indications from recent Space Shuttle experiments have been very promising[3]. These space-grown crystals were larger and better ordered than those grown under normal laboratory conditions. They were brought back to Earth gently, which may be an essential condition for improved results; the dramatic accelerations of unmanned re-entry may itself introduce disorder (or worse). Even if microgravity proves to be efficacious, however, the cost will keep it far beyond the means of the average laboratory.

Back on Earth, more attention is being given to examining the factors which affect successful nucleation and growth. Robots in the form of automated machines are available both commercially and home made, and will make it much easier to explore the possible conditions for growth. They will be needed no matter how successful analytical tools such as electron microscopy prove — no method for improving the size or internal order of crystals can succeed without initial crystals to examine, and trial-and-error will always be required to provide the first glimmer of crystallization.



a, Step pattern generated by a defect on the face of a lysozyme crystal. The spiral, it is thought, originates at the site where a screw dislocation intersects the surface. The irregular edges (T) are tears in the replica of the surface. *b*, Clearer view of a spiral pattern; steps generated at a defect spiral out and are roughly parallel at large distances. The dark spots and uneven transmission of the replica are due to imperfections in the preparation. Scale bar, 0.5 μm. (From ref. 2.)

Still, many frustrated crystallographers are left wondering why their crystals will not grow large enough or well-ordered enough, or grew yesterday but will not grow today. Durbin and Feher's first results show the 'normal' process of growth very clearly as it is revealed both on the outer surfaces and within the body of the grown crystal. The two basic processes by which material is added to a growing crystal face seem to be the same for proteins as were seen over 20 years ago in paraffins. In both, new molecules are added to a step on the growing face propagating it across the underlying layer until it cannot extend any further, usually at the edge of the face. At high degrees of supersaturation, new steps are produced by nucleation of growing layers on top of existing layers. At low degrees of supersaturation this process is too slow and is outstripped by the growth from step dislocations.

To visualize how such a mechanism works, imagine layers of card, one on top of the other, representing the molecular layers of a perfect crystal, the top one being a crystal face. Cutting through these layers from their edges to their centres results in new edges which extend half way in to the crystal. If the layers are distorted where the cut ends in the centre, so that the cut edge of each layer joins the cut edge of the upper layer adjacent to it, instead of the layer at the same level as before, a step forms across half the crystal face. This step can grow, but as it grows it must spiral around the central dislocation. This is just the spiral growth pattern that Durbin and Feher observe (see figure), and which in other micrographs can be seen propagating through the body of the crystal. Of more significance to those whose crystals won't grow large enough, Durbin and Feher believe that they can also see where steps collide and jam, resulting in cessation of growth.

Many attempts have been made to encourage crystals to grow larger. Etching back the stopped surface and starting again in fresh protein solution has been tried with some success[4]. Again, however, the methods used have been almost all of the trial-and-error variety. Treading in the steps of Durbin and Feher, crystallographers can now settle down to analysis of their own material in the real hope of understanding the events that are increasing the disorder or stopping the growth of their crystals. ☐

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1. McPherson, A. *Preparation and Analysis of Protein Crystals* (Wiley, New York, 1982).
2. Durbin, S.D. & Feher, G. *J. molec. Biol.* **212**, 763–774 (1990).
3. DeLucas, L.J. *et al. Science* **246**, 651–654 (1989).
4. Thaller, C. *et al. Meth. Enzymol.* **114**, 132–135 (1985).

EPIDEMIOLOGY

Radon and the risks of cancer

Julian Peto

AT the end of April, Denis Henshaw and colleagues[1] published their finding of significant correlations between average indoor radon levels in different countries and the incidence of leukaemia and other cancers in children, and of myeloid leukaemia, kidney cancer and melanoma in adults. Last week, again in *The Lancet*, they announced a similar correlation for prostatic cancer[2]. Significant regional correlations have also been reported between indoor radon and acute myeloid leukaemia in Britain[3] and Canada[1].

Two letters in this week's *Lancet* add fresh grist to the debate. Using data from their atlas of regional leukaemia and lymphoma rates in England and Wales[4], Ray Cartwright's group describes several significant correlations of cancers — including acute myeloid leukaemia in adults and acute lymphoblastic leukaemia in children — with indoor radon[5]. Scientists from the National Radiological Protection Board (NRPB) however cast doubt on Henshaw's data and conclusions[6]. They observe that both records of cancer incidence and estimates of average radon exposure are of questionable accuracy for some countries, and that the international correlations between adult cancer rates and radon are lower and not statistically significant when the less reliable data are excluded. The correlation for childhood cancers is not reduced in this re-analysis. But the authors point out that the NRPB risk-assessment model for childhood leukaemia predicts that the effect of radon is undetectably small, and argue that in view of this conflicting evidence such correlations constitute only tenuous evidence of cause and effect.

Inconclusive findings

Epidemiological studies on radiation and childhood cancers, particularly leukaemia, have produced a series of intriguing but inconclusive findings in recent years. The discovery in 1983 of a cluster of childhood leukaemias near the nuclear reprocessing plant at Sellafield led first to the report of the advisory group chaired by Douglas Black[7], and then to the establishment of the Committee on Medical Aspects of Radiation in the Environment (COMARE). The three reports that COMARE has already published confirm that there were unusual clusters of childhood leukaemia near both Sellafield and Dounreay (the only other British reprocessing plant), and that the incidence was also slightly increased in the area surrounding Aldermaston[8-10]. This last observation was supported by an analysis from Richard Doll's group[11] of leukaemia mortality below the age of 25 in different regions of England and Wales, which revealed a small but statistically significant increase in areas close to a nuclear installation.

By this stage the various associations seemed to constitute good evidence of a link, but it was difficult to attribute them to known discharges. Sellafield had emitted much larger amounts of radioactivity than Dounreay, yet the excesses in the local leukaemia rates were similar, and levels of environmental radiation in areas surrounding most other nuclear plants were so low that they seemed unlikely to be the direct cause of any detectable excess. Various other studies provided evidence of unexplained clustering in areas distant from nuclear plants, and the leukaemia death rate below age 25 throughout England and Wales was found to be greater in areas where a high proportion of the population were of high social class[11]. This could not be accounted for by the difference in leukaemia mortality between social classes, which is minimal in the latest national statistics, and raised the suspicion that something quite different might be involved.

This suspicion was confirmed last year by Leo Kinlen. Kinlen demonstrated[12] that isolated communities sometimes suffer a marked increase in childhood leukaemia after a sudden population influx, and he suggested that an anomalous pattern of childhood exposure to an infectious agent could be the cause. The communities near Sellafield and Dounreay are extreme examples of such population change.

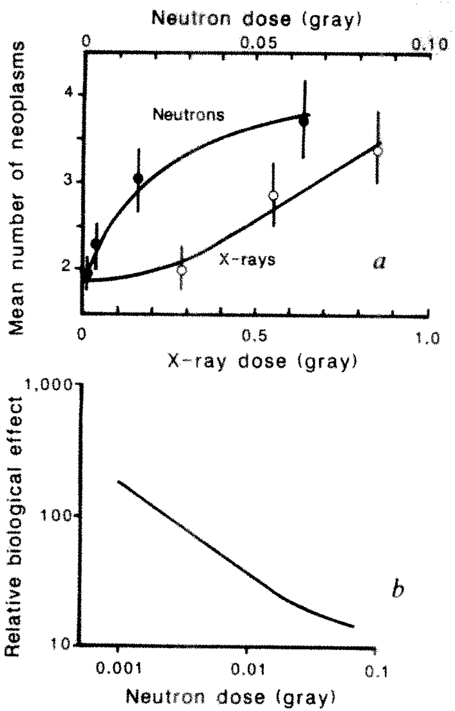
The growing belief that the apparent associations with nuclear installations were more likely to be related to early infections than to radiation was, however, unexpectedly challenged three months ago. Martin Gardner and his colleagues[13] found that all four of the fathers of children with leukaemia living near Sellafield for whom radiation records could be traced had suffered higher radiation exposure than most other workers at Sellafield. Although the association is based on only four cases, it is highly statistically significant, and is the more worrying because the lifetime exposures of the men involved were within approved international limits. These leukaemias achieve statistical significance by two completely independent criteria: first, the high local incidence, which would not have been significantly elevated if these four cases had not occurred; and second, the high external exposure of their fathers to gamma radiation compared with other employees at Sellafield.

The Gardner study thus seems to provide quite strong evidence that the children of the most heavily exposed workers at Sellafield suffered an increased risk, although a direct effect of paternal exposure to gamma radiation is only one of several possible explanations. As well as chance, other explanations include exposure to mutagenic chemicals, ingestion of alpha-emitting radionuclides and the possibility that such exposures can activate latent leukaemogenic viruses[14]. The most heavily exposed workers' families might also be the most mobile, and hence particularly liable to the effect discovered by Kinlen. Studies are now underway on the children of similarly exposed workers at other plants.

Risk estimates

Henshaw's hypothesis that radon causes a significant proportion of childhood leukaemia and adult myeloid leukaemia (and possibly some other cancers) appears to contradict conventional risk estimates, but such estimates are based on a number of questionable assumptions, particularly for internal high-LET (linear energy transfer) emitters and childhood cancers. The conventional 'quality factor' is 20 for high-LET radiation such as alpha particles or neutrons, which means that their actual energy transfer to tissue must be multiplied by 20 to calculate the biologically equivalent dose of gamma radiation. Henshaw and his colleagues point out that high radon levels are associated with increased external gamma radiation, so any correlation with radon is likely to be due in part to the gamma dose, and they conclude that the most plausible interpretation of their findings is that the quality factor for myeloid leukaemia induction by radon daughters is higher than 20 but less than 180. A value of 20 is certainly too low at low doses, although too high at high doses, in some (although not all) experimental systems, reflecting the very different dose–response curves sometimes exhibited by high-LET and low-LET radiation[15] (see figure, over). The assumption that high- and low-LET radiations can be treated as equivalent by using a single conversion factor is a recognized weakness in conventional risk assessment. In certain respects these exposures appear to act as qualitatively different carcinogens, and their effects should really be modelled separately.

A further complication is the internal distributions of different radionuclides. These distributions have not been well characterized, but elements certainly vary in their tendency to concentrate in particular tissues, and presumably in their effect on the haemopoietic stem cells from which leukaemia develops. In principle, the distribution of radon is easier to model than that of other radionuclides — it is chemically inert and its radioactive daughters are short-lived, so its pathway



a, Dose–response curves for mammary tumour induction in female Sprague–Dawley rats, exhibiting downward curvature for neutrons and upward curvature for X-rays at low doses. (Redrawn from ref. 15.) The dose scales for neutrons and X-rays differ by a factor of 10. *b*, The authors' estimate of the corresponding relationship between relative biological effect (RBE) and dose shows that the RBE exceeds 100 at 0.001 Gy, and suggests that it may be even higher at lower doses.

in the body is determined largely by solubility.

Predictions of lifetime cancer risk to the general population resulting from very low exposures, such as mesothelioma from asbestos in buildings or leukaemia from average radon levels, are usually so low (about 1 in 100,000 or less) that they cannot in principle be confirmed epidemiologically. Moreover, they are based on dose estimates which for certain types and routes of exposure have not been monitored adequately. A model of carcinogenesis that makes untestable predictions from inadequate measurements is more akin to social than natural science, and an epidemiologist confronted with an apparent contradiction between the predictions of radiobiological theory and the incidence of human cancer is bound to suspect that the human data are more reliable. The extraordinary if tiny local clusters of childhood leukaemia at the only nuclear reprocessing sites in Britain, the weaker but still significant excess around Aldermaston, Gardner's evidence that the children of heavily exposed Sellafield workers are at high risk of leukaemia, and Henshaw's correlations between childhood cancer and radon are examples of such contradictions. Some of these observations may be due to chance, and some may be completely unrelated to radiation, as Kinlen has demonstrated; but there must remain a substantial probability that some are due to unpredicted carcinogenic effects of radiation. In view of Gardner's findings, one possibility is that childhood cancer is caused by paternal exposure to radon.

At a practical level, Henshaw's findings, even if confirmed, merely reinforce the generally accepted conclusion that indoor exposure to high levels of radon is a considerable environmental hazard. The NRPB estimate that as many as 2,000 lung cancers may be caused by radon each year in Britain[6] provides sufficient grounds for reducing exposure where practicable, irrespective of any added risk for childhood cancer and the other adult cancers for which Henshaw reports significant correlation with radon exposure. The central question is whether or not these correlations constitute strong evidence of cause and effect, and in the absence of clear epidemiological evidence or a much better quantitative understanding of the mechanism of cancer induction the answer must depend largely on one's prior belief in the causal link. Henshaw and his colleagues have been studying the internal distribution of alpha-emitting radionuclides, and conclude that the dose to the red bone marrow and fetus from absorbed radon and its daughters may be substantial[1].

Any scientist would be excited to discover epidemiological correlations that appear to support a hypothesis that emerged from painstaking laboratory studies, and it would be unreasonable to complain because Henshaw and his colleagues are convinced that the relationships they have observed are real. Subsequent research may show that they are wrong. But they have assembled a *prima facie* case for an unexpected, intriguing and testable hypothesis that must certainly be pursued. ☐

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1. Henshaw, D. L., Eatough, J. P. & Richardson, R. B. *Lancet* **335,** 1008–1012 (1990).
2. Eatough, J. P. & Henshaw, D.L. *Lancet* **335,** 1292 (1990).
3. Lucie, N. P. *Lancet* **II,** 99 (1989).
4. Cartwright, R. A., Alexander, F. E. McKinney, P. A. & Ricketts, T. J. *Leukaemia and Lymphoma: An Atlas of Distribution within Areas of England and Wales 1984–1988* (Leukaemia Research Fund, London 1990).
5. Alexander, F. E., McKinney, P. A. & Cartwright, R. A. *Lancet* 2 June 1990.
6. Butland, B. K., Muirhead, C. R. & Draper, G. J. *Lancet* 2 June 1990.
7. Black, D. *Investigation of the Possible Increased Incidence of Cancer in West Cumbria*, (HMSO, London, 1984).
8. COMARE *First Report* (HMSO, London, 1986).
9. COMARE *Second Report* (HMSO, London, 1988).
10. COMARE *Third Report* (HMSO, London, 1989).
11. Cook–Mozaffari, P. J. *et al. Br. J. Cancer* **59,** 476–485 (1989).
12. Kinlen, L. J. in *Medical Responses to Effects of Ionising Radiation* (eds Crosbie, W. A. & Gittus, J. H.) 272–278 (Elsevier Applied Science, London, 1989).
13. Gardner, M. J. *et al. Br. med. J.* **300,** 423–429 & 429–434 (1990).
14. Evans, H. J. *Nature* **345,** 16–17 (1990).
15. Shellabarger, C. J., Chmelevsky, D. & Kellerer, A. M. *J. natn. Cancer Inst.* **64,** 821–833 (1980).

DAEDALUS

Just keep rolling!

MANY urban roads are permanently choked by parked cars, and ruthless methods are often deployed to discourage them. Daedalus now proposes a method which is some sort of ultimate in efficiency and ruthlessness. He recalls those intriguing liquids with nonlinear viscosity, like silicone putty, starch suspensions and mixtures of sharp sand and water ('quicksand'). Under slow steady pressure they flow easily; but rapid impact locks them up into a momentary solid. A suspension of sand in tar is a common road surface, and DREADCO's chemists are now seeking formulations or additives to endow such a mixture with extremely nonlinear viscosity.

A road of DREADCO's 'Quicktarmac' will not inconvenience travelling vehicles. Under the rapid impact of their fast-moving wheels it will act as a stable and reliable solid. But a motorist trying to park on it will find himself in deep trouble. It will rapidly yield to the steady pressure of his static tyres. His car will sink in, and will soon be firmly stuck. Ideally, it should sink completely out of sight, punishing the errant motorist by total loss of his vehicle and freeing that section of road for legitimate traffic again.

But such a great depth of Quicktarmac would be prohibitively expensive. A thin layer of the material on a conventional concrete base will be almost as good. A parked vehicle will merely sink in up to its axles. Thus entrapped, it can never acquire enough velocity to solidify the Quicktarmac and climb onto the surface again; it can only plough slowly and stickily through the material until it reaches the limit of the no-parking zone. An official release vehicle could perhaps free it by violent impact from behind, giving it sufficient instantaneous velocity to ride up again onto the road surface; but most offenders would rather crawl away, crimson with fury and embarrassment, without such aid.

This elegant principle can clearly be extended. Modified formulations of Quicktarmac could discourage kerb-crawling motorists or enforce minimum-speed regulations on link highways. Pedestrians are also strongly motivated not to linger dangerously on the road, but to cross briskly and purposefully.

Even more useful, Quicktarmac is self-repairing. Scars and potholes will fill themselves in by slow spontaneous flow, while trash and rubble sink out of sight. By the same token, however, traditional road camber would flatten out, and a road laid on a gradient would slowly flow downhill. While one might design such a road as a series of shallow steps separated by cross-ribs, Daedalus acknowledges that his new technology is best kept on the level.

David Jones

What use is “euxeny”?

Sir—Rasmont is not the first person to feel uncomfortable about using the term ‘altruism’ in the context of animal behaviour, even if he is the first to suggest the neologism “euxeny” as an alternative[1]. The problem with his suggestion is that many will interpret his term as meaning that biological insights into the evolution of cooperation are simply not relevant to ‘real’, human altruism.

In practice, the problem hinges on the definition of the latter, not the former. In animal behaviour we have every justification for ignoring motives and defining altruism purely in terms of its objective consequences as any act which enhances the reproductive success of the recipient at a cost to that of the altruist. There are good reasons for thinking that there are three — and almost certainly only three — types of altruistic behaviour: kin altruism, where the sacrifice of the actor benefits copies of the gene for altruism in the recipient to an extent greater than the cost to itself[2]; reciprocal altrusim, where the actor's sacrifice for the benefit of the recipient is reciprocated by the recipient[3]; and induced altruism, where an actor performs an altruistic act through error, deceit or coercion[4,5]. Examples of the latter would be birds who raise the offspring of cuckoos, believing them to be their own. On the objective definition of altruism above, they must be seen as acting altruistically because they have incurred a considerable cost to their own reproductive success and enhanced that of the cuckoo.

As Rasmont implies, what makes ‘real’, human altruism controversial is the belief that it is not motivated by any kind of self-interest. Of course, neither is it in the case of the apparently odd, residual category of induced altruism. The birds who raise cuckoos have absolutely no self-interest in doing so. Nevertheless, they have been deceived, and so we seem to have to conclude that it is our consciousness of acting without self-interest which defines ‘true’, human altruism.

But the fact that politicians routinely claim to act in the public interest, rather than in their own self-interest, is not a reason why voters should be expected to believe them. On the contrary, Anna Freud's classic psychoanalytic study of the motives involved in human altruism concluded that “It remains an open question whether there is such a thing as a genuinely altruistic relation to one's fellow men, in which the gratification of one's own instinct plays no part at all . . .”[6]. To separate “euxeny” from human altruism, as proposed by Rasmont, would really make it too easy for the politicians, and my guess is that if behavioural scientists were not human beings themselves they would have long since realized that attempts to defend subjective definitions of human altruism are completely futile. Perhaps this is because there is a little of the politician in all of us.

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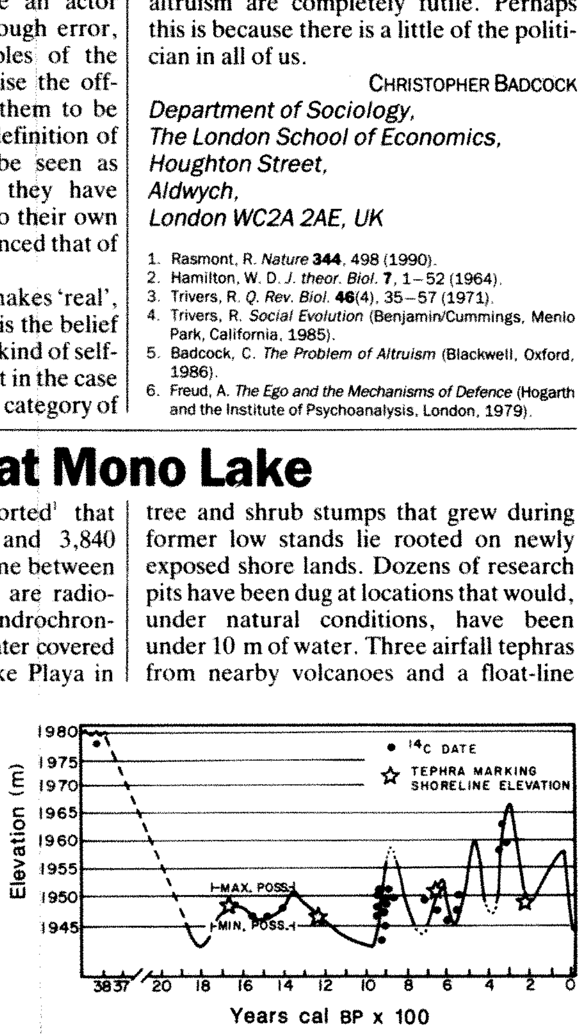
1. Rasmont, R. *Nature* **344**, 498 (1990).
2. Hamilton, W. D. *J. theor. Biol.* **7**, 1–52 (1964).
3. Trivers, R. *Q. Rev. Biol.* **46**(4), 35–57 (1971).
4. Trivers, R. *Social Evolution* (Benjamin/Cummings, Menlo Park, California, 1985).
5. Badcock, C. *The Problem of Altruism* (Blackwell, Oxford, 1986).
6. Freud, A. *The Ego and the Mechanisms of Defence* (Hogarth and the Institute of Psychoanalysis, London, 1979).

Past climate at Mono Lake

Sir—Enzel *et al.* have reported[1] that some time between 4,000 and 3,840 calendar years BP and some time between 522 and 313 cal BP (all dates are radiocarbon dates calibrated by dendrochronology), a substantial body of water covered the now-desiccated Silver Lake Playa in the Mojave Desert. Mono Lake, 380 km to the north, is by contrast a perennial body of saline-alkaline water which can provide a palaeoclimatic record covering a wide range of conditions, from the wettest to the driest[2].

The artificial recession of Mono Lake in recent decades has made accessible previously drowned evidence of past lake-level fluctuations. At least four generations of tree and shrub stumps that grew during former low stands lie rooted on newly exposed shore lands. Dozens of research pits have been dug at locations that would, under natural conditions, have been under 10 m of water. Three airfall tephras from nearby volcanoes and a float-line of pumice blocks from a sub-lacustrine volcanic eruption have made it possible to reconstruct lake margins at the time of eruptions.



Late Holocene fluctuation curve for Mono Lake.

Most importantly, the drop in lake level has forced the three largest tributary streams to incise their deltas to depths of up to 10 metres. The walls of these cuts, each more than 1,000 metres long, show alternating sequences of transgressive and regressive deltaic sediments replete with plant detritus and interspersed with tephras of known age. Individual transgressive and regressive units can often be traced directly to a strand line that marks their terminal elevation. This evidence, combined with twenty-six radiocarbon dates, provides a direct and high-resolution record of the late Holocene fluctuations of the level of Mono Lake[3].

In addition to its prominent peaks at approximately 3,770 and approximately 300 cal BP, which appear to correspond to the hydric episodes at Silver Lake Playa, in the late Holocene, Mono Lake underwent five other decades-long transgressions. Analyses using Vorster's hydrological model[2] indicate that the effective inflow to the Mono Lake groundwater basin required to explain the highest of these transgressions was in excess of 134 per cent of the modern (1937–79) average value. Not apparent from the Silver Lake record are prolonged periods when the climate was dryer than it is now, represented at Mono Lake by recessions and low stands, the most extreme of which occurred in response to decades-long effective inflows averaging less than 68 per cent of the modern value.

Corroboration can be found in the Bristlecone pine tree-ring record not only for the Silver Lake stands[1], but also for the late Holocene fluctuations of Mono Lake[2]. Even more intriguing is the apparent correspondence between the Mono curve and the record of DeVries-type solar variations[4,5]. This suggests that decades to century-scale climatic changes such as those reflected in the fluctuations of Mono Lake and other lakes of the western Great Basin may be related to such solar events as the Maunder, Sporer, Wolf and mediaeval sunspot minima, and the mediaeval and Roman sunspot maxima[2,3].

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1. Enzel, Y., Cayan, R.D., Anderson, R.Y. & Wells, S.G. *Nature* **341**, 44–47 (1989).
2. Stine, S. thesis, University of California (1987).
3. Stine, S. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (in the press).
4. Stuiver, M. & Quay, P. *Science* **207**, 11–19 (1980).
5. Eddy, J. *Climatic Change* **1**, 173–190 (1977).

Hot solution

Sir—Nelson's report (*Nature* **344**, 115–116; 1990) that cables and instrumentation in remote locations can be protected by the application of Tabasco sauce and silicone sealant is a reminder that those who tend orchards also have problems with masticatory and/or curious animals, and that there is a product which is much more potent, easier to apply and (if bought in bulk) cheaper than Tabasco. This is Hot Sauce Animal Repellent (Miller Chemical and Fertilizer Corp., Hanover, Pennsylvania), which is a 2.5 per cent solution of capsaicin extracted from capsicum peppers. I have found this to be effective in protecting young almond trees, although I have heard anecdotal reports of deer that have apparently acquired a taste for this spicy fare. But Nelson's addition of silicone sealant seems to me to be an improvement.

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Methods of leprosy prevention

Sir—Bloom does not discuss the use of leprosy vaccines in his commentary article[1] "Vaccines for the Third World". Is this because vaccines are not the right approach to the eradication of leprosy? The simple, practical, alternative measure is to treat the patients with dapsone, kill the bacteria and prevent the spread of the disease. Where this has been done and measurements carried out there has been a dramatic decline in the disease, for example, in northern Zaria state, northern Nigeria, the prevalence of leprosy fell from 46 per 1,000 in 1953 to 1.6 per 1,000 in 1967. In the village of Igabi it fell over the same period from 67 per 1,000 to 2 per 1,000 (ref. 2).

I have previously presented[3] evidence for a decline in incidence. Since then, dapsone resistance has increased but has not caused a resurgence of the disease at least in Katsina Province, northern Nigeria, where the prevalence in 1987 was 2 per 1,000 down from 39 per 1,000 in 1951 (ref. 4). The introduction of multi-drug therapy may lessen the frequency of resistance to dapsone.

There are still areas where leprosy is endemic and where patients do not have access to this therapy. Even in those areas where it is available the decline in incidence is rarely recorded. In my opinion, failure to extend this treatment and ensure proper evaluation while trying to develop an effective vaccine is irresponsible, especially as the presence of the antibody against the human immunodeficiency virus may make people more vulnerable to leprosy[5].

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Bloom replies—Although dapsone therapy has made a significant contribution to the treatment of leprosy in the past, WHO has recommended multidrug therapy with three antibiotics (including dapsone) for several reasons. Multidrug therapy is more effective, has thus far blocked the emergence of drug-resistant organisms that is one of the main problems with single drug treatment, prevents relapses after treatment and can reduce the duration of treatment from a lifetime to a few years or perhaps less. Serious efforts are being made by WHO, the International Leprosy Organization and many endemic countries to extend multidrug therapy to as many patients as possible. Just under half of registered patients worldwide are now receiving this type of treatment. Finally, because of the long latency of the infection, transmission of leprosy frequently occurs before treatment can begin.

The point of developing a vaccine is to prevent infection and disease. It was an omission in my commentary not to mention that four candidate anti-leprosy vaccines are currently in clinical trials.

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1. Bloom, B.R. *Nature* **342**, 115–120 (1989).
2. Crawford, C.L. *Lepr. Rev.* **40**, 159–163 (1969).
3. Crawford, C.L. *Nature* **254**, 168–170 (1975).
4. Waaldijk, K. *Lepr Rev.* **60**, 59–61 (1989).
5. Meeran, K. *Br. med. J.* **264**, 364–365 (1989).

What are sisters good for?

Sir—An important and prevalent error surfaced in a recent Scientific Correspondence[1], in which Conover questioned why female/female pairings among birds are so rare, given that females in such pairs generally produce fertile eggs. Conover correctly pointed out that pairs of unrelated females would have to produce twice as many offspring as male/female pairs to attain the same average reproductive success per female. His mistake was to assert that sister/sister pairs must produce only 33 per cent more young than male/female pairs. This would be true only under very improbable circumstances, and then only for one of the two sisters.

Suppose, for example, that female/female pairs fledge four young (two per female) and male/female pairs fledge three young, on average per breeding season. Thus, if homosexually paired females are unrelated, each gets credit for two young fledged. If the females are sisters, then according to Conover, their average inclusive fitness should equal that of females paired heterosexually (three young, in this example). This is the result one obtains by applying the erroneous "simple weighted sum" definition of inclusive fitness[2,3] in which individuals are credited with all of their own offspring plus all of their relative's offspring (devalued by relatedness).

Hamilton[4] defined inclusive fitness as that portion of individual fitness not attributable to the positive or negative influences of others, plus any harms or benefits conferred on others (weighted by relatedness). The appropriate question is whether a female pairing with her sister benefits her sister sufficiently to offset the cost of not pairing with a male, ($b \geq c/r$). If both sisters could produce the same number of offspring by pairing with an unrelated female, then there would be no benefit to pairing with a sister. In fact, each would suffer an inclusive fitness decrement due to the cost her sister would incur by not pairing with a male. Only in extremely asymmetrical relationships would one of the sisters in a homosexual pair get credit for as many genetic replicates as a female in a male/female pair (again, using the above hypothetical numbers of fledglings). Specifically, if a given female could fledge a total of four young with either an unrelated female or her sister, but her sister's only reproductive opportunity were to pair with her, then her inclusive fitness would equal three young if she paired with her sister versus two young if she paired with an unrelated female. Although theoretically possible, the situation is not likely to occur in nature.

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1. Conover, M.R. *Nature* **342**, 624–625 (1989).
2. Grafen, A. *Nature* **298**, 425–426 (1982).
3. Grafen, A. in *Behavioural Ecology: An Evolutionary Approach* 2nd edn (eds Krebs, J.R. & Davies, N.B.) 62–84 (Blackwell, Oxford, 1984).
4. Hamilton, W.D. *J. theor. Biol.* **7**, 1–52 (1964).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. ☐

Ice-age dust and sea salt

SIR—Harvey[1] has estimated the climatic effect of increased tropospheric aerosols during the last glacial maximum (LGM). Attributing to the present tropospheric aerosol a globally averaged optical depth, τ, of 0.13 (at 550-nm wavelength) and a 2-3-K surface cooling, Harvey rather more than doubles τ during the LGM and finds a further cooling of 2-3 K.

The ice-core evidence he cites concerns only two aerosol components: continental dust, which is measured by the insoluble particles Al or Ca^{2+}; and marine sea salt, which is measured by Na^+. Together, dust and sea salt form the 'coarse mode', or supermicrometre, fraction of today's atmospheric aerosol, with sources, sinks and optical properties distinct from the fine, or submicrometre, mode.

Because these two modes vary independently in the atmosphere (which the ice-core record itself dramatically confirms[2]), Harvey's modelling exercise properly constitutes an estimate of the LGM cooling resulting from dust and sea salt only, and should therefore have begun with an estimate of the optical depth due solely to the coarse mode, τ_{coarse}, in the present-day climate. Note that the studies cited for confirmation of his control model were each concerned with the total tropospheric aerosol, not just the coarse mode fraction.

Early investigators (see ref. 3) recognized that the tropospheric aerosol mass is more or less evenly divided between primary particles, mainly dust and sea salt, and secondary particles (those produced *in situ* from gases), mostly sulphates. It was later demonstrated that these primary and secondary components have very different sizes[4,5] and, consequently, very different solar extinction efficiencies[6], the fine-mode sulphates being about six times more efficient per unit mass. Thus, a reasonable estimate, in agreement with limited direct observations, is that the coarse mode causes about 15 per cent of τ. (It will cause an even smaller fraction of aerosol-induced cooling since larger particles scatter sunlight much more into the forward direction.)

Taking this estimate as uncertain by a factor of 2 and assuming that current τ falls somewhere between 0.1 and 0.2 (ref. 7), we can estimate τ_{coarse} to be within the range 0.01 to 0.06. Thus, Harvey's calculation, based on a control model τ of 0.13, should be considered as an extreme upper limit of the climatic forcing due to ice-age dust and sea salt. Because Harvey's estimated LGM cooling of 2-3 K is simply proportional to the value of τ used in his control model, it should be reduced by a factor of at least two and possibly ten.

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1. Harvey, L. D. *Nature* **334**, 333–335 (1988).
2. Legrand, M. R., Delmas, R. J. & Charlson, R. J. *Nature* **334**, 418–420 (1988).
3. Jaenicke, R. *J. aerosol Sci.* **11**, 577–588 (1980).
4. Whitby, K. T. *Atmos. Envir.* **12**, 135–159 (1978).
5. Clarke, A. D., Ahlquist, N. C. & Covert, D. S. *J. geophys. Res.* **92**, 4179–4190 (1987).
6. Willeke, K. & Brockmann, J. E. *Atmos. Envir.* **11**, 995–999 (1977).
7. Toon, O. B. & Pollack, J. B. *J. appl. Meteorol.* **15**, 225–246 (1978).

Chance marsupial relationships

SIR—Thomas *et al.*[1] interpret their evolutionary tree, based on 12S ribosomal RNA gene sequence data for the marsupial wolf (*Thylacinus cynocephalus*) and six other marsupials, as evidence that the thylacine does not lie outside the Australian cluster of marsupial lineages. The strength of this evidence depends on the confidence we can have in their tree topology (their Fig. 2), but a comparison of the cladistic analysis of the observed 12S ribosomal RNA data with similar randomized data sets suggests that their tree is not significantly different in its degree of cladistic structure, from cladograms produced 'by chance alone'.

The length of a cladogram (the smallest number of character state changes implied by the tree of minimum length) is a measure of cladistic structure. It reflects the extent to which co-variation of the characters allows hypotheses of common ancestry, as represented by sister-group relations in the cladogram, to explain shared character states among the taxa[2,3]. Because cladistic algorithms will impose some cladistic structure even on data consisting of random numbers, it is informative to ask whether a given data set has more cladistic structure than would be expected by chance.

The original cladistic structure can be compared to the structure revealed by artificial data sets in which each original character is taken in turn and its observed character states are randomly reassigned, without replacement, to the taxa. A Monte Carlo study (repeated computer simulations using random numbers) compares the observed data to many such randomized sets.

The Monte Carlo test for the 12S ribosomal RNA data focuses on the relationship of thylacines to other accepted monophyletic groups of marsupials. The two taxa in the *Dasyuridae* and the two in *Phalangeridae* are each combined into a single family-level taxon, to avoid testing for structure in the data due simply to the monophyly of these groups. Thus, five marsupial taxa are included in the data set. Because the outgroup status of *Philander* (*Didelphidae*) is well supported[4], its character states are held constant; it is used as an outgroup taxon in the analysis of the randomized sets.

Characters (ribosomal RNA sites) that are invariant or which differ by a single character state among the five taxa are ignored; these will contribute constant amounts of character change over all data sets, and will not affect the results of the test. The reduced data set therefore has seven phylogenetically informative characters for the five taxa (see table). Cladistic analysis recovers the same topological relationships between the five families as reported by Thomas *et al.*; the tree has nine steps.

One of the randomized data sets from the Monte Carlo study is also shown in the table (data in parentheses and bold type). From a total of 99 randomized sets, a tree of the same length as, or shorter than, that of Thomas *et al.* is found nine times. A rough estimate of the significance is the proportion of all trees (including the original) having nine steps or fewer, that is 0.10.

The results of this Monte Carlo test are surprising. They imply that if the 12S ribosomal RNA nucleotides at each of the seven sites had been randomly assigned to taxa, there would have been a reasonable chance (about 1 in 10) that the cladogram produced would have had as much struc-

CHARACTER STATES AT PHYLOGENETICALLY INFORMATIVE SITES

Taxon	Site 1	2	3	4	5	6	7
Didelphidae	G(**G**)	C(**C**)	C(**C**)	T(**T**)	A(**A**)	C(**C**)	C(**C**)
Thylacinidae	A(**A**)	C(**C**)	T(**T**)	A(**T**)	A(**T**)	T(**C**)	T(**C**)
Dasyuridae	G,A(**A**)	C(**A**)	T(**T**)	A(**T**)	A(**T**)	T(**T**)	T(**T**)
Peramelidae	G(**G**)	A(**A,T**)	C(**T**)	T(**A**)	T(**A**)	C(**C**)	C(**T**)
Phalangeridae	A(**G,A**)	A,T(**C**)	T(**C**)	T(**A**)	T(**A**)	C(**T**)	C(**C**)

Nucleotides at each of the seven informative sites (results of a Monte Carlo simulation in parentheses and bold type). For two of the sites in each array, one of the taxa is assigned two different nucleotides. The necessary extra character state change within the taxon is ignored in calculating tree length. The taxon assigned 'A,T' at site 2 is parsimoniously interpreted as having ancestral state 'A', and that assigned 'G,A' at site 1 as having that ancestral state implying the smallest number of character state changes for a given tree.

ture as that in Thomas *et al.*'s cladogram. I conclude that the 12S ribosomal RNA data constitute insufficient evidence to determine the phylogenetic relationships among these taxa, and in particular that the data do not exclude the possiblity that the thylacine is an outgroup to the Australian lineages.

Our more formal presentation of the Monte Carlo test and its application to other taxonomic data sets[5] supports the recent view that, for both animals[6] and plants[7], the degree of character convergence and reversal found in molecular data sets matches that of morphological data. This property, together with the large number of phylogenetically uninformative characters found in most molecular data sets, suggests that the apparent wealth of molecular information for phylogenetics is frequently illusory.

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THOMAS *ET AL.* REPLY—Faith's randomization test, which is similar to one recently published by Archie[8], demonstrates the absence of any significant departure from randomness for the 12S ribosomal RNA gene sequences taken as a whole. But for testing if particular groups of taxa in a tree are monophyletic or not, the bootstrapping approach is more powerful[8]. For the particular question addressed in our paper[1], the latter test gives significant support for the thylacine–dasyurid clade. Other portions of the tree, such as the position of the bandicoot *Echymipera*, are not well supported by the 12S ribosomal RNA gene data when tested by bootstrapping.

Furthermore, confidence in the existence of the thylacine–dasyurid clade is enhanced by the quantitative immunological results obtained with albumin[9] and by the finding that the ratio of transitions to transversions observed in the cytochrome *b* gene is higher for comparisons of Australian marsupial carnivores with each other and the thylacine than for all comparisons of these marsupials with others[1]. In an attempt to achieve even higher confidence, we intend to obtain more sequences from the thylacine and extant marsupials.

As for Faith's view that molecular traits are not superior to morphological ones for building reliable genealogical trees, we refer readers to the case of the orang utan's position among hominoid primates. Despite more than a century of intensive morphological analysis, no statistically significant support emerged for any of the three following trees, in which: the orang utan is less akin than humans are to African apes; the orang utan is more akin to humans than African apes are; or the orang utan is more akin than humans are to African apes[10,11]. Yet studies of DNA reveal 121 traits supporting tree A, 8 supporting tree B and 10 supporting tree C — a highly significant result[11]. A possible reason for Faith's pessimistic view is that the cases to which he refers are for small proteins whose resolving power is inherently low when used to probe deep branches in genealogical trees.

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1. Thomas, R.H., Schaffner, W., Wilson, A.C. & Paabo, S. *Nature* **340**, 465–467 (1989).
2. Farris, J.S. *Syst. Zool.* **28**, 483–519 (1979).
3. Faith, D.P. *Cladistics* **5**, 235–258 (1989).
4. Clemens, W.A., Richardson, B.J. & Baverstock, P.R. in *Fauna of Australia* Mammalia (eds Walton, D.W. & Richardson, B.J.) 527–548 (AGPS, Canberra, 1989).
5. Faith, D. P. & Cranston, P. S. *Cladistics* (in the press).
6. Wyss, A.R., Novacek, M.J. & Mckenna, M.C. *Molec. Biol. Evol.* **4**, 99–116 (1987).
7. Syvanen, M., Hartman, H. & Stevens, P.F. *J. molec. Evol.* **28**, 536–544 (1989).
8. Archie, J.W. *Syst. Zool.* **38**, 239–252 (1989).
9. Lowenstein, J.M., Sarich, V.M. & Richardson, B.J. *Nature* **291**, 409–411 (1981).
10. Wilson, A.C. *Scient. Am.* **253**(4), 164–173 (1985).
11. Wilson, A.C. *et al.* in *The Hierarchy of Life (eds Fernholm, B. et al.)* 407–419 (Elsevier, Amsterdam, 1989).

Papain and related proteins

SIR—Recent contributions to Scientific Correspondence[1-3] have addressed the structural similarity of the 111K antigen of the malaria parasite *Plasmodium falciparum* to cysteine proteinases in the amino-acid sequence region containing Cys 22 and Cys 25 in papain. The fact that Cys 585 of the 111K antigen is analogous to Cys 22 of papain[2,3] rather than to the catalytic site Cys 25 (ref. 1), for which the analogous residue is Ser 588, prompted Mottram *et al.* to suggest[3] that the 111K antigen might represent an evolutionary intermediate between cysteine and serine proteinases.

In considering the possibilities for variation in the catalytic-site regions of cysteine proteinases and structurally related proteins, our isolation of a protein in a metastable configuration from the latex of *Carica papaya* in which first, the sulphur atom of Cys 25 probably exists as a disulphide with the sulphur atom of either Cys 22 or Cys 63; and second, one or other of these residues contains a free thiol group, may be of interest.

In papain, the side chains of Cys 25 and His 159 form an interactive system with nucleophilic character in weakly acidic solution modulated by binding interactions and electrostatic field effects[4,5]. The $-\mathrm{S}^-/-\mathrm{Im}^+\mathrm{H}$ ion-pair component of this interactive system is probably the catalytic device responsible for the nucleophilic and general acid attack on the scissile bond of a substrate[6]. The side chains of Cys 22 and Cys 63 form one of the three disulphide bonds of the papain molecule, and analogous features are known to exist in other cysteine proteinases.

The reactivity of the thiol group of Cys 25 in active papain towards the two-hydronic-state reactivity probe[7] 2,2′-dipyridyl disulphide (2-Py-S-S-2-Py) is abnormally high in acidic media and maximal at pH 3.8. The abnormal reactivity derives from the coexistence at pH 3–4 of significant concentrations of (Cys 25)$-\mathrm{S}^-$/(His 159)-$\mathrm{Im}^+\mathrm{H}$ ion pair and the highly electrophilic 2-Py-S-S-2-$\mathrm{Py}^+\mathrm{H}$ cation[6].

In marked contrast, the thiol group of the metastable papaya latex protein ('propapain') exhibits more normal reactivity characteristics typical of a 'bare' thiol group that is not part of a Cys–His interactive system, with maximal reactivity in alkaline media and negligible reactivity in acidic media[8,9]. Treatment with a low molecular mass mercaptan such as cysteine converts the inactive, thiol-containing, metastable protein into catalytically active papain, with retention of thiol stoichiometry and with marked change in thiol reactivity characteristics to those typical of cysteine proteinase catalytic sites[6].

The results of kinetic[8] and labelling[9] experiments support the view that the metastable protein is a structural isomer of papain containing a different disulphide bond (Cys 22–Cys 25 or Cys 25–Cys 63), and that its conversion to papain involves thiol-promoted thiol-disulphide interchange to produce the free thiol group of Cys 25, which forms the catalytic-site ion pair with His 159.

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1. Higgins, D.G., McConnell, D.J. & Sharp, P.M. *Nature* **340**, 604 (1989).
2. Eakin, A.E., Higaki, J.N., McKerrow, J.H. & Craik, C.S. *Nature* **342**, 132 (1989).
3. Mottram, J.C., Coombs, G.H. & North, M.J. *Nature* **342**, 132 (1989).
4. Brocklehurst, K. *et al. Biochem. J.* **256**, 543–555 (1988).
5. Kowlessur, D. *et al. Biochem. J.* **259**, 443–452 (1989).
6. Brocklehurst, K., Willenbrock, F. & Salih, E. in *Hydrolytic Enzymes* (eds Neurberger, A. & Brocklehurst, K.) 39–158 (Elsevier, Amsterdam, 1987).
7. Brocklehurst, K. *Meth. Enzym.* **87C**, 427–469 (1982).
8. Brocklehurst, K. & Kierstan, M.P.J. *Nature new Biol.* **242**, 167–170 (1973).
9. Kierstan, M.P.J., Baines, B.S. & Brocklehurst, K. *Biochem. Soc. Trans.* **10**, 172–173 (1982).

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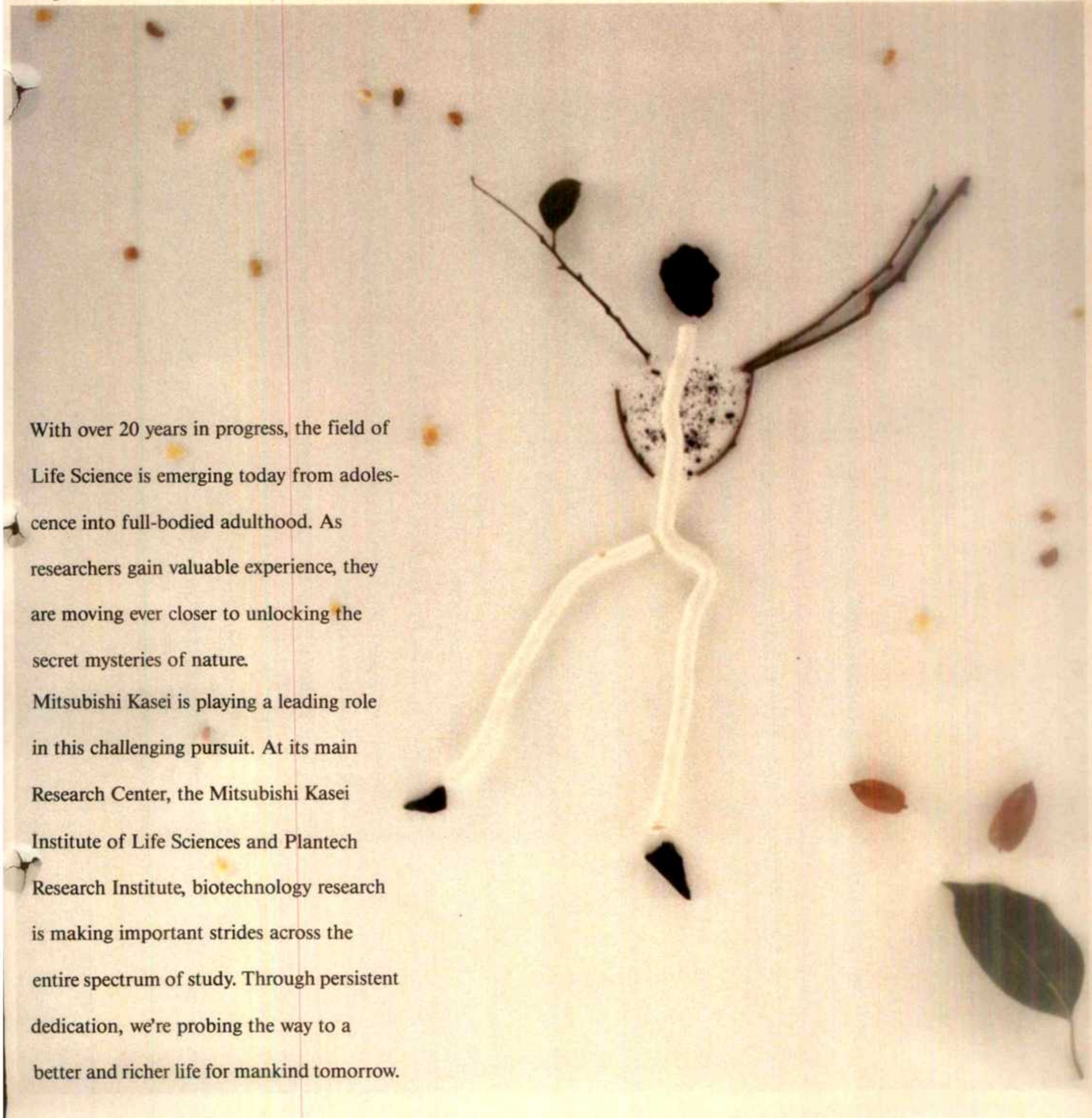

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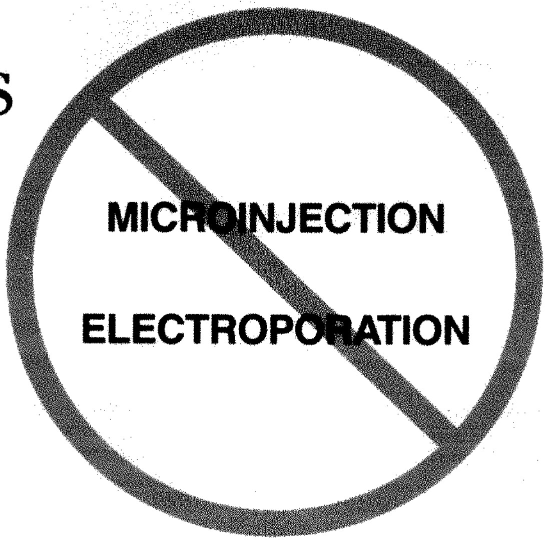

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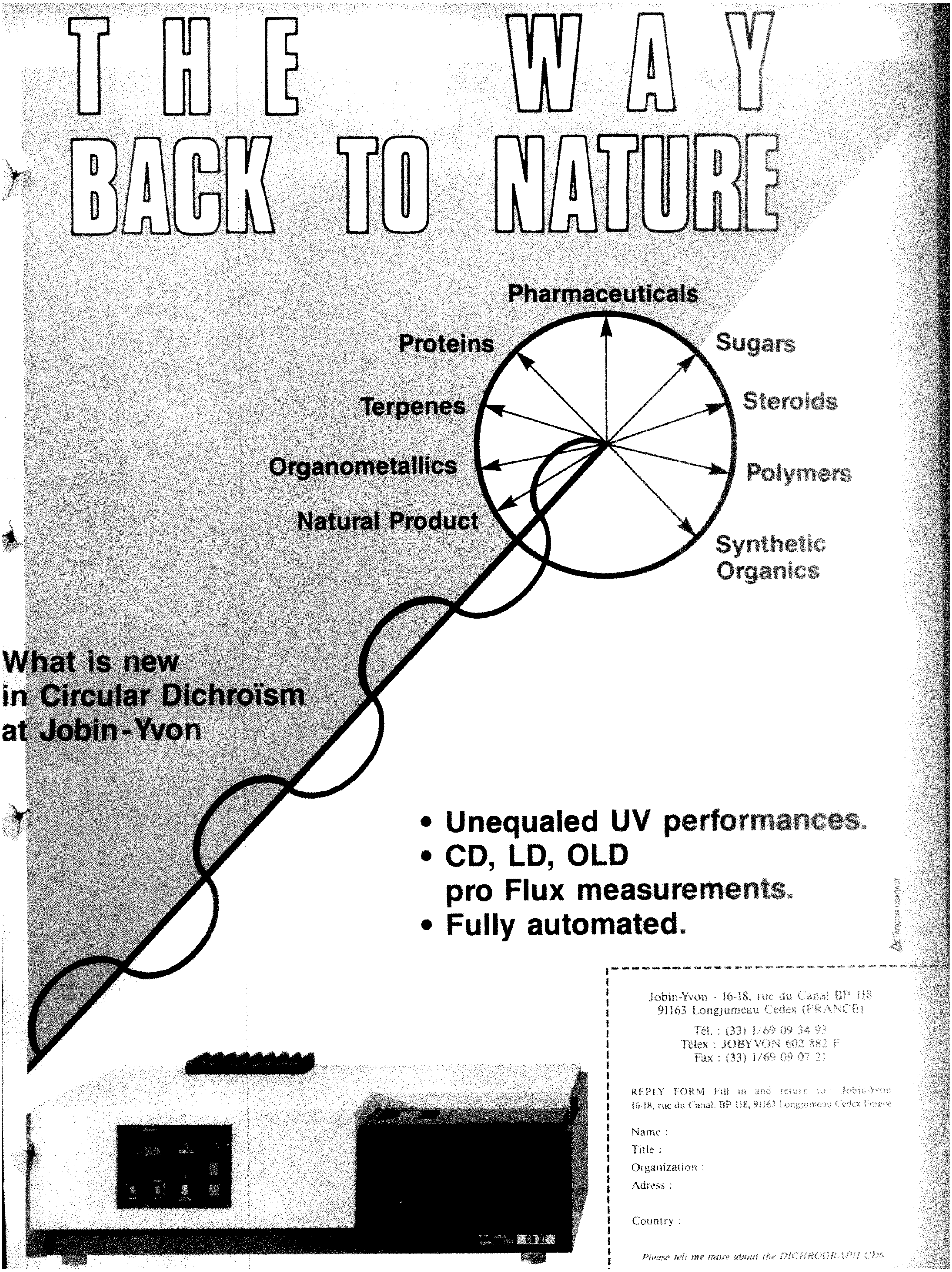

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A thousand and one Eves

J. S. Jones

The Search for Eve. By Michael H. Brown. *Harper & Row: 1990. Pp.357. £16.95.*

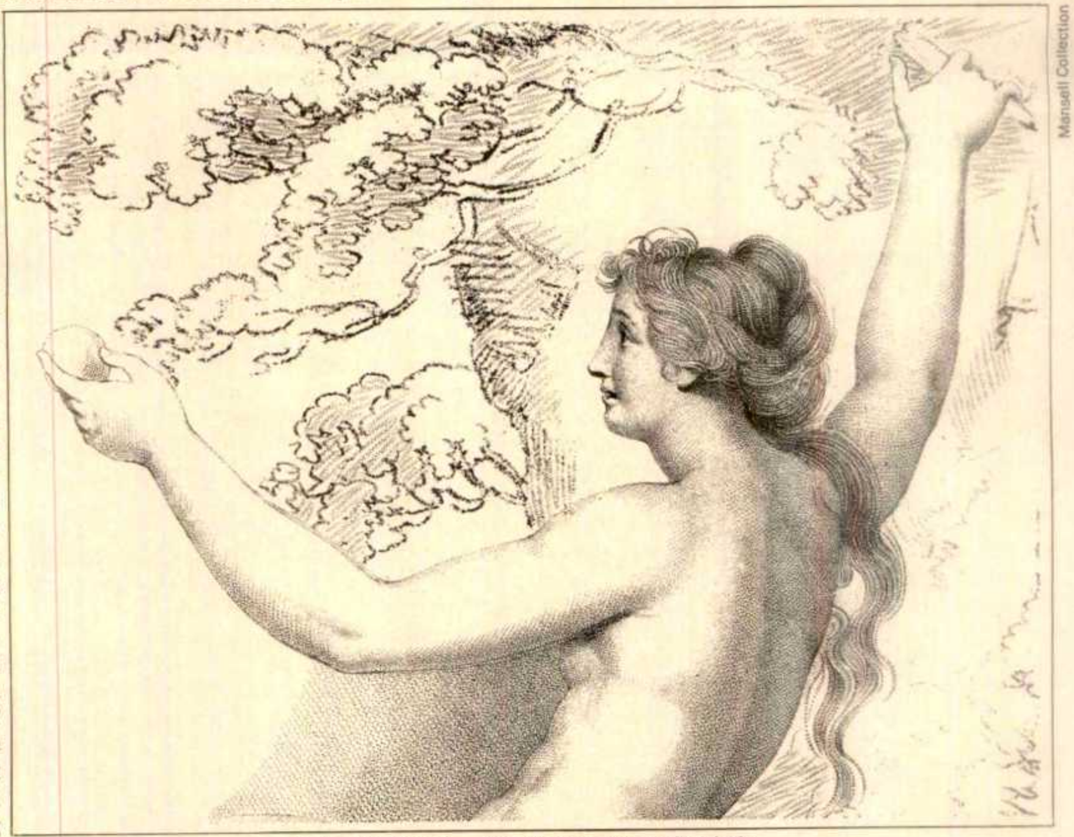
MITOCHONDRIAL DNA, like Jewishness, is inherited down the female line. As we each have one mother, two grandmothers and so on, then we all must share a remote female ancestor in common. What is true for Jews is also true for Joneses: surnames travel down the generations through males and — except for names which originate more than once — everyone with a particular name can trace it to a common male forebearer. The ur-Jones lived a few hundred years ago; the Ultimate Jewish Mother some centuries before that.

Brown's book is a journalist's account of the search for the mother of us all; the mitochondrion which lay at the root of the human family tree and within the cells of a woman who inevitably became known as "Eve". It is a racy tale about the human race and about the quarrels of those who study it. As usual, the science is more interesting than the scientists. Does it really matter that one proponent has "piercing gelid eyes" while another is a "demure earth mother"? Admittedly, though, there are few fields which can boast papers written from prison where the author is serving time for poisoning the judge who gave him a drugs sentence. Palaeoanthropologists seem to make up for a lack of fossils with an excess of fury, and this must now be the only science in which it is still possible to become famous just by having an opinion. As one cynic says, in human palaeontology the consensus depends on who shouts loudest.

The genetics of human ancestry has also led to some raised voices, with plenty of accusations of incompetence and fraud. Although there are some worrying exceptions it does seem that for most genes, including those of the mitochondrion, the ancestral populations are in Africa (or, possibly, in the Middle East). From there, say the geneticists, the first modern humans spread through the world, driving the Neanderthals to extinction without mating with them. This is heresy to many students of the fossil record, who see what appear to be intermediate forms in other parts of the globe. They mock the idea of 'killer humans', spreading like killer bees and wiping out the "klutzing lame-brain Neanderthals" — to quote one of the many clumsy phrases in this book — as they went.

This mockery is misplaced. First, it is hard to see how pre-human populations thousands of miles apart could independently evolve into the same new species. In addition, invaders — rabbits, grasses, fruitflies or squirrels — can spread at an astonishing rate. They often displace their relatives without interbreeding; a process which, ironically enough, is manifest in the patterns of mitochondrial genotypes in the killer bees introduced into Brazil 30 years ago. There is nothing intrinsically implausible about the 'out of Africa' view of human evolution.

The fossil record can at least tell us exactly who was where and when, but its incompleteness means that it is very likely that no fossil hominid yet found is on the direct line of descent to modern humans. As a molecular biologist quoted in this book says, we know that modern genes came from our ancestors, but we can never be sure that any fossil left a descendant. Fossils are, above all, evidence for the fact of evolution rather than for how it happened. But although genetics can tell us a lot about the patterns of relatedness of living populations, it can do little more than speculate about the time and place when they split. What comes out of a genetic model often depends on what assumptions go in: to take an unusually rigorous view of molecular evolution — that of the creationist Duane Gish — the fact that man and watermelon are both 97 per cent water does not necessarily mean that they have a recent ancestor in common.



Pick of the bunch — Eve, the mysterious first lady.

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Nothing has caused more argument than the date when various lineages parted from each other, or when "Eve" might have flourished. New fossil discoveries mean that man has been getting younger every year, and there are now no major disagreements about the date of the separation of the human and the ape family trees. The date of our mitochondrial great-grandmother is much more contentious. The speed at which her descendants diverged from each other depends on two things: the rate of mutation — which can be measured — and the size of the evolving populations, which cannot. Any estimate as to how many early humans there were and hence the date of the mitochondrial mother must be a wild guess. Indeed, the latest estimates of Eve's birth date are between fifty and five hundred thousand years ago, estimates so wide as to be almost meaningless.

Our ancestor's biblical nickname does not, of course, mean that she was then the only female. There were thousands of others, but the accidents of family life, with some mothers having no daughters and emigrants taking with them only a few of the genes of their homeland when they left, mean that most mitochondrial lineages have been lost. Unfortunately, and *in spite* of all the publicity about the discovery of the Earth mother, Eve was a statistical artefact.

However, her biography makes a good story. The latest idea is that the spread of one mother's genes accompanied the origin of language; a theory reported here as "scientists claim woman had the first word". This and new work on the Y chromosome means that perhaps we will soon see a book reporting not only where Eve met Adam but what they said to each other. ☐

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P. V. E. McClintock

Reflections on Liquid Helium. By E. L. Andronikashvili. *AIP: 1990. Pp. 317. £45, $60.*

THE non-appearance of invited Soviet speakers at international conferences — an all-too-familiar feature of the international scientific scene during the several decades of the cold war — and the resultant relative isolation of Soviet science inevitably led to inefficiences, infelicities and lost opportunities. One thinks, for example, of the numerous cases of wasteful repetition of work already completed and published on the other side of the iron curtain, of delayed recognition for important achievements, and incorrect attributions of priority in discovery.

Much of the excellent Soviet science of this period has consequently been shrouded in a certain mystery, for those in the West, with many of the chief personae becoming familiar in name only, and years afterwards. This is especially true in the case of the (enormous) Soviet contributions to low-temperature physics, and in particular to the understanding of superfluid helium. The name (but not the person) of Andronikashvili is well known to every low-temperature physicist on account of his famous experiment in which he measured directly the relative proportions of the two components of liquid helium below its superfluid transition temperature at 2.17 kelvin: the normal component (which behaves like an ordinary liquid) and the superfluid component (which displays a variety of exotic properties such as inviscid, frictionless flow).

What Élevter Luarsabovich Andronikashvili achieved by managing to complete his *Reflections on Liquid Helium* before his death last year has been, in effect, to "take the lid off" half a century of Soviet low-temperature physics, revealing not only the circumstances of the particular experiment which made him famous, but also the personalities, relationships, arguments, intrigues, motivations and the many difficulties involved in practicing experimental scientific research at the highest level within a closed society. In content, the book represents a kind of scientific autobiography but, as Russell Donnelly points out in his foreword, it is written much more in the style of a novel.

The story opens with Andronikashvili at the age of 29, already established in a chair at the University of Tbilisi in Georgia, but in great trepidation agonizing about whether or not to dare to apply for permission to spend a year in Kapitsa's famous Institute for Physical Problems in Moscow. He was eventually persuaded by his sister-in-law Viva to do so, and the decision changed his life. It also, to a significant extent, modified the future of low-tempertaure physics. The tale continues with vivid descriptions of Andronikashvili's time in the institute, his enforced return to Tbilisi, and his subsequent (successful) fight to escape from his administrative and political responsibilities for enough of the time to maintain contact with the world of research. Such a succinct summary of the book must make it appear tame but, in reality, it is anything but that.

Most of Andronikashvili's *Reflections* is devoted to discussions of his scientific and social relationships with colleagues, rivals, friends and visitors, and with his hosts in the numerous departments and institutes in the West that he was eventually able to visit. The story is very much a human one, and is told in large part though quotations of direct speech. The reader may wonder about the accuracy with which conservations are being remembered, in some cases, almost 50 years after the event; but such reservations seem petty in the face of the liveliness, vigour and intrinsic interest of the narrative itself. It provides a fascinating account, from the viewpoint of a participant, of how discoveries are really made — including the errors, arguments, misapprehensions and false starts — as opposed to the tidy *post hoc* rationalization that is usually all that is embodied in a scientific paper. The book will thus be of interest to historians and philosophers of science, as well as to low-temperature physicists.

There are scores of entertaining anecdotes, and there are critical and often highly amusing descriptions of the (literally) hundreds of people whom Andronikashvili met or worked with in the course of his scientific career. The latter include a galaxy of well-known names, such as Kapitsa, Landau, Bogolyubov, Peshkov, Khalatnikov, Frenkel, Shal'nikov, Bohr, Peierls, Allen, Feynman, Bardeen, Abraham, Hall and Vinen — to name only a tiny sample. Andronikashvili's character studies of close associates, for example Kapitsa and Landau, are highly illuminating. In other cases, where his trenchant remarks are based on slighter evidence (such as a single meeting at a conference) they can occasionally seem a little unfair. The persons portrayed are usually highly recognizable, though, and the book will certainly provide a source of considerable amusement to the victims' colleagues and friends.

One of these victims of an Andronikashvili vignette was recently heard to remark good-humouredly: "but he was a pompous little man himself, anyway". Perhaps he was — I never had the privilege (or, possibly, the liability) of meeting him — but he was clearly much more besides. Inevitably, *Reflections* tells us at least as much about its author as about the people he set out to describe. Andronikashvili was clearly a complex and interesting character with coexisting, seemingly contradictory, traits: of impetuous enthusiasm, coupled with meticulous attention to detail; of brusqueness and intolerance, but great personal kindness; of pride and a perhaps overly pronounced sense of hierarchy, mercifully punctured by a well-developed sense of humour. He was also a distinguished scientist who has left a permanent mark on low-temperature physics and on science in Georgia.

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New Journals

This year *Nature's* annual new journals review supplement will appear in the issue of 11 October. Publishers and learned societies are invited to submit journals for review, taking note of the following criteria:

- Journals which first appeared after June 1988, and which issued at least four separate numbers by the end of April 1990, will be considered for review. The deadline for submission is the end of June.
- Journals covering any aspect of science are eligible, although those dealing with clinical medicine, engineering and pure mathematics are excluded, as are publications of abstracts.
- Frequency of publication must be at least three times a year.
- The main language used must be English. Translation journals in English are eligible.

When submitting journals for review, please send at least four different issues (the first, the most recent and any two others) of each title as soon as possible to: Book Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, UK or 1137 National Press Building, Washington DC 20045, USA.

Right and wrong

Stephen Lock

Beyond Baby M. Edited by Dianne M. Bartels, Reinhard Priester, Dorothy E. Vawter and Arthur L. Caplan. *The Humana Press Inc: 1990. Pp. 288. $29.50 ($34.50 export).*

Philosophical Ethics in Reproductive Medicine. Edited by David R. Bromham, Maureen E. Dalton and Jennifer C. Jackson. *Manchester University Press: 1990. Pp.261. £35.*

THE public perception of bioethical problems is that they arrive on the scene, fully grown, like Venus from the sea. Surrogate motherhood began to be discussed in the mid 1980s; by May 1988 more than 600 children had been born by this method in the United States. Surrogacy seems unlikely to disappear as a contentious issue, and other current anxieties include the definition of death and the extension of life, embryo research, the allocation of resources (particularly high versus low technology), and the validity of scientific advance for its own sake.

Public concerns make good conference material, but it seems a pity that the organizers of these affairs seem unable to resist recording their deliberations in print. As reviewers never tire of pointing out, the result is inevitably a mixed bag. Some work is original, other findings are not; the papers are of variable quality, even more obviously so when the discussions are included; and some of the content is out of date by the time of publication.

Unhappy families — Mary Beth Whitehead (left), surrogate mother of Baby M. Elizabeth and William Stern (centre and right): potential adoptive mother and biological father.

All these comments apply to *Beyond Baby M* and *Philosophical Ethics in Reproductive Medicine*, one from the United States, the other largely British, both based on conferences held two years ago. Sensibly, the US volume omits the discussions and its main thrust is on surrogate motherhood. This practice is not only tedious and costly (averaging $20,000–35,000 per case) but involves a legal minefield, as shown in the case that gives the book its title. The salient details were well documented in the media: although the Sterns, a childless couple from New York, signed a surrogacy parenting agreement, the biological mother, Mary Beth Whitehead, changed her mind after the birth of baby Melissa. Three court cases resulted in the decision that William Stern and Mary Whitehead are Melissa's legal parents, both with rights and responsibilities towards her. Given its reputation as a leader in both family law and medical ethics, the forthrightness of the New Jersey Supreme Court was hardly surprising. The real evil was paid surrogacy: "Whatever idealism may have motivated any of the participants the profit motive predominates, permeates, and ultimately govern the transaction" — an aspect recognized early in Britain, where commercial surrogacy was banned in 1985.

The commentaries in this book tease out many of the unappreciated facets of the Baby M case. There is the white/blue collar tension, with the Sterns paying $60,000–70,000 on legal expenses and private detectives to track the Whiteheads down to their hiding place. There were also family problems — with Mrs Stern having mild multiple sclerosis (not initially revealed to Mrs Whitehead) and Mr Whitehead an unsteady employment record and unresolved alcohol problems — not to mention the subsequent divorce of the Whiteheads, with the settlement that he will share in any book or movie royalties. Thus, not surprisingly, a feminist commentator, Barbara Katz Rothman, considers that society is encouraging "production standards" in pregnancy, thinking of the woman herself not as a person but as the container for another, often more valued, person — yet at $1.57 per hour for her service, the woman is hardly well rewarded.

Disappointingly, in the sole contribution on surrogacy in the British volume, Edgar Page declines to address the feminist perspective, as he does to tease out the argument that if priority is given to the birth mother as against the genetic mother then it is difficult to maintain that mother and father have equal parental status. In fact, with six sections, *Philosophical Ethics in Reproductive Medicine* ranges too widely, and the discussions would have been better devoted to printing Iain Chalmers's lecture, published in a journal not readily available, and to providing overviews ("We will leave readers to enjoy tracing these overlaps for themselves", the editors say; well I for one, didn't). To be sure, there are some first-rate accounts: Gordon Dunstan's characteristically wise introduction on the moral status of the embryo, commenting on the remarkable advance in understanding the issues raised by *in vitro* fertilization; Alex Campbell's comparison of withholding neonatal care in the United Kingdom and the United States with case-by-case analyses in the former and an increasing tendency to decision-making by ethics committees in the latter; and Richard Lilford's cogent argument that informed comment is now part of decision theory.

Women will run considerable risks for their unborn children. Lilford cites a woman 20 weeks' pregnant found to have cancer of the cervix who was told that immediate treatment sacrificing the pregnancy would carry an 80 per cent chance of complete cure. Asked how delaying treatment until the baby had a 95 per cent chance of survival would reduce her prognosis, he estimated 10 per cent. The woman decided to continue the pregnancy, against her husband's wishes; he urged immediate treatment. (Fortunately, the outcome was successful for both mother and baby, but Lilford argues that even if the mother had died the decision would still have been correct.)

Nowhere is the evanescence of such meetings better illustrated than in the "emergency" discussion of fetal brain cell transplants. The first operation for Parkinson's disease in Britain had taken place in Birmingham only days beforehand and inevitably the proceedings were tentative, ignoring several issues now known to be crucial (including the unresolved one of efficacy). This is not to criticize the discussants (though were the proceedings worth printing?) but the issue does illustrate the need for some central body to anticipate such developments. The House of Commons in Britain now has no forum for discussing these (though the Lords retains its select committee on science and technology) and it seems extraordinary that Britain has no clear, authoritative source of advice on ethical and policy issues. Several other countries have national bioethics committees — Australia, Denmark, France and Italy — and an outstanding example was the US President's Commission, disappointingly discontinued by President Reagan. It has been left to private bodies to discuss forming such a committee in the United Kingdom, and at a Nuffield Foundation Conference in April it was agreed that such a committee should involve wide consultation using a discussion document. There is a good case for imitating the evolution of the committee on *in vitro* fertilization in Britain, which has progressed from a voluntary through an interim to an eventual statutory basis. ☐

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- Also just published is *The New Reproductive Technologies*, edited by M. McNeil, I. Varcoe and S. Yearley. Publisher is Macmillan, price £35 hbk, £12.99 pbk.

Pulling together

Malcolm Irving

Molecular Mechanisms in Muscle Contraction. Edited by J. M. Squire. *Macmillan: 1990. Pp. 327. £55.*

'MOLECULAR mechanism' has a special meaning in muscle research, because muscle is composed of an array of molecular machines that use the free energy of ATP hydrolysis to produce force or motion. At the heart of the machine are the proteins myosin and actin, the main constituents of the two types of filament which slide past each other when muscles shorten. The subject of this multi-author volume is the structure of actin and myosin filaments and the mechanism of interaction between them. The most popular model for this interaction, the 'rowing' or 'swinging' crossbridge model, proposes that myosin projections transiently bind to the actin filament and rotate about the point of attachment to produce relative filament motion. To quote from the preface, "a major theme of this book is to present the different kinds of structural and mechanical evidence in favour of such a process".

Structural studies dominate the book. An introductory chapter on the basic structural elements of muscle is followed by two more on the structure of the actin monomer and filament. Unfortunately, these were written before the determination of the high-resolution structure of the actin monomer from actin–DNase crystals, which has been discussed at recent meetings and is expected to be published soon. This is likely to have a major impact on the field, and may challenge some of the conclusions and speculations advanced in this book. But the discussion of actin structure here will not be completely superseded, and its main conclusion, that large-scale movements between domains of the actin monomer are possible, could be important in the mechanism of filament sliding. In the swinging crossbridge model, the actin filament is usually considered as a passive track, but this may turn out to be an oversimplification.

There are four chapters devoted to studies of the conformations and movements of the myosin crossbridges in muscle fibres, using X-ray diffraction, electron microscopy, fluorescence and spin probes. It is difficult to compare or link these results, as the techniques report on different structural features and have been applied in different conditions and preparations. (For example, the electron microscopy refers almost exclusively to the flight muscle of the giant waterbug *Lethocerus*, and one of the chapters on X-ray diffraction concentrates on fish muscle.)

Nevertheless, a common conclusion is that the myosin crossbridge may take up more than one conformation while it is attached to the actin filament, as predicted by the swinging crossbridge model. The structures of these attached states, and their relation to the process of force generation, remain poorly defined and it is at least arguable that these studies have not yet provided firm evidence either for or against the model. An alternative approach is discussed here by Harrington *et al.*, who present a clear and comprehensive account of the evidence for a force-generating mechanism based on a helix–coil transformation in part of the tail of the myosin molecule. Currently the best argument against this hypothesis is the observation that the isolated head fragment of myosin can both move actin filaments and produce the normal force per head, in the absence of the tail. Unfortunately, the chapter seems to have been written before some of these observations were published, so the reader is deprived of the authors' reply to this apparently powerful argument.

Structural studies have shown two attached states of the myosin crossbridge, often called 'weak' and 'strong', by analogy with the binding affinities of the corresponding actin–myosin states in solution. These are discussed in detail in a lucid and thoughtful chapter by Brenner, who also reviews the relationship between solution studies of the actin–myosin ATPase and muscle fibre. The model that emerges is considerably more complicated than the original swinging crossbridge scheme of the 1970s. The contrast between current mechanochemical and structural models for crossbridge function emphasizes how little is yet known of the structures of the transient intermediate states responsible for filament sliding, even at the low resolution of the available structural methods. The low resolution is itself a serious limitation, as might be inferred from the chapter reviewing nuclear magnetic resonance studies of muscle proteins. This chapter discusses the general principles underlying conformational changes in contractile proteins, stressing their dynamic nature and using the role of troponin C in muscle regulation as an example. The corresponding studies of actin–myosin are in a state of relative infancy, but are likely to receive new impetus from knowledge of the crystal structure of actin.

This book does not provide a comprehensive treatment of its subject, and some important approaches are conspicuously absent, notably the more recently developed areas such as molecular genetics and motility studies on isolated actin–myosin. The reference base for the articles extends only to 1988. Each chapter presents a rather personal view of the problem, and these views do not fit together into a neat consensus. On the other hand, each group of authors has the space to develop its ideas in detail and depth, and several of the resulting reviews will, I believe, be of lasting value to those working in the field.

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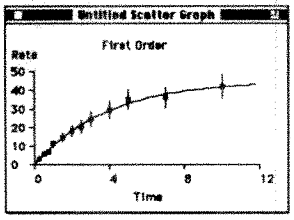
New in paperback

- Two books on dinosaurs have just been issued in paperback. *Digging Dinosaurs*, by John Horner and James Gorman, was reviewed in *Nature* **338**, 552; 1989. Published by Harper and Row, price $8.95. *Dinosaur Plots* (and other intrigues in natural history), by Leonard Krishtalka, is published by Avon Books, price $8.95. For review see *Nature* **338**, 673; 1989.
- *Life Among The Scientists*, by Max Charlesworth *et al.*, is subtitled "an anthropological study of an Australian scientific community". Reviewed in *Nature*'s Autumn Books issue last year (**342**, 312; 1989), publisher is Oxford University Press, price £8.95.
- Also just published by Oxford is *The World Within the World* by John Barrow, a wide-ranging study of the laws of nature. Price is £8.95. For review see *Nature* **336**, 288; 1988.



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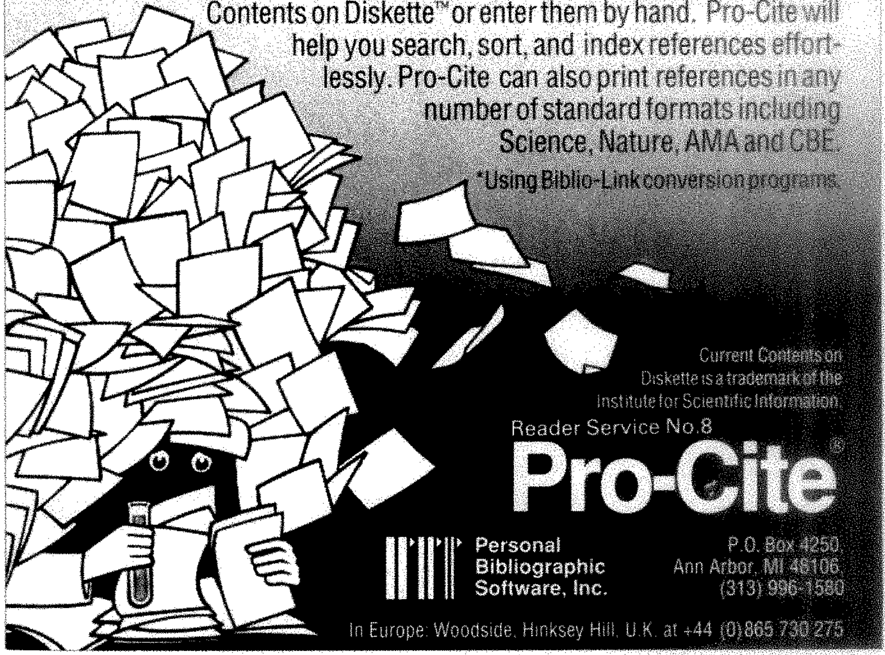

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Applications of electron cooling in atomic, nuclear and high-energy physics

Helmut Poth

The introduction of electron cooling has opened up new possibilities in atomic, nuclear and particle physics. Now many newly constructed ion storage rings are making use of this technique.

BEAMS of charged particles are used commonly to probe the fundamental structure of matter and to investigate interactions between elementary particles. As one studies ever smaller scales, the probes must be increasingly energetic and sensitive. Antimatter projectiles, in particular antiprotons ($\bar{p}$), give access to hitherto inaccessible domains. Because of their opposite charges, one can accelerate counter-rotating beams of particles and antiparticles simultaneously in a single accelerator ring and bring them into collision. The centre-of-mass energies reached in colliding-beam experiments are much higher than those from collisions of projectiles with a target at rest. For instance, one would need to collide antiprotons with energies of 5×10^{12} eV (5,000 GeV) with hydrogen nuclei in a fixed target in order to match the energy of 1,000-GeV colliding $p\bar{p}$ beams. Consequently, the accelerator would have to be 50 times bigger than the collider.

Electron cooling provides a means for focusing such particle beams and reducing their energy spread. These refinements are far more than an experimental nicety: they open up a whole range of investigations that would be otherwise impossible. Electron-positron (e^-e^+) beams are used in collider experiments, but proton-antiproton beams provide higher energies. Antiprotons have to be created artificially. Enough energy is available to produce them in high-energy proton-nucleus collisions, but the yield is low; they have to be accumulated before colliding with protons, if reasonable reaction rates are to be obtained. Moreover, antiprotons so produced are spread out in energy and space, so a single pulse injected into an accumulator ring will fill it up. The pulse must be compressed in order that many pulses may be accumulated in the ring. In a reference frame moving with the beam velocity (the orbiter frame), the energy spread and the divergence correspond to velocity distributions parallel and perpendicular to the beam direction, with corresponding temperatures amounting to millions of degrees. Compression cannot be achieved by standard ion-beam optics, because Liouville's theorem states that the volume occupied by particles in phase (position and momentum) space cannot be reduced by the action of conservative forces (such as those that govern the motion of particles in accelerators and storage rings). According to this principle, the phase-space volume is like a balloon filled with air: squeezing the balloon in one region makes it expand elsewhere. The only way to reduce the volume of the balloon is by cooling the gas. This can be achieved by cooling the material of which the balloon is made, and relying on collisions of the gas with the balloon wall to transmit the cooling to the gas. This is viable as long as the particles do not interact destructively with the wall of the container. But in the case of high-energy beams, wall collisions would result in destruction of the container and projectile, so such a scheme is not feasible. How, then, can the particle beam be cooled? The alternative is to mix it with a cold gas which cools it through direct collisions between the gas particles and the antiprotons. Annihilation has to be avoided, so one needs a gas of particles that interact only electromagnetically with the antiprotons—electrons or positrons would be suitable. Electrons with temperatures of a thousand degrees or so can be obtained easily. This cooling of a beam using a cold electron gas was first proposed by Budker[1].

Charged particles can also be cooled very efficiently in a different way. Moving charges induce electric fields in antennas when they pass close by, the field being related to their velocity. By feeding the induced signal to capacitor plates through which the charges pass further downstream, a retardation or an acceleration can be imposed on the particles that is dependent on their initial velocity. This method, called stochastic cooling, was invented by van der Meer[2]. The signals are picked up at random from ensembles of charged particles, whence the term 'stochastic'. Stochastic rather than electron cooling is more appropriate for cooling high-energy antiprotons[3,4], but electron cooling is used to improve antiproton beams at low energy, and opens up new possibilities in the study of antinucleons and their interaction with ordinary matter. A further important application of electron cooling is as a means of compressing stored beams of light and heavy ions to permit spectroscopic investigation of highly charged ions. Initial studies of this sort used protons[5-7], but the method has now also been applied to antiprotons[8] and light ions[9], yielding intriguing results.

New possibilities for physics

Cooled ion beams are small in diameter and divergence and have a sharp energy distribution, in these respects resembling laser beams. These properties are essential for high spatial and energy resolution, allowing precision experiments with rare species such as antiprotons. Collision and annihilation of antiprotons and protons at low energy, can give information about the interior structure of (anti) nucleons. Such collisions can be initiated with the particles almost at rest, because their opposite electric charges result in a mutual attraction which binds them together in a heavy hydrogen-like atom (a $\bar{p}p$ bound state). On disintegration, their constituent quarks and antiquarks rearrange to form other hadronic particles such as pions. Spectroscopy of these annihilation products yields information on how the quarks and antiquarks are bound together and how they interact with each other. An accurate study of these aspects requires a well defined initial situation (for example, a low energy spread and a well defined interaction angle, which can be obtained by cooling the antiproton beam.

To slow cooled antiprotons it is possible to attach a positron to form antihydrogen. The mass of the antiproton and its gravitational interaction can be measured with high accuracy when antiprotons are confined in electromagnetic traps; electron cooling can help to make capture efficient and to lower the antiprotons temperature. A comparison between the atomic spectroscopy of particle and antiparticle systems enables one to test with great precision the validity of fundamental symmetry relations in particle physics.

For atomic and heavy-ion physics, the perspectives are no less spectacular. Ion sources can produce only partly ionized heavy ions. For instance, to strip uranium atoms of all electrons, they must be accelerated to velocities greater than the velocity of the K electron (~70% of the speed of light) and passed through a thin foil. The passage through the foil causes the beam to 'blow up'; cooling can compensate for this, resulting in a small, monochromatic beam of fully stripped ions that can be used for the high-precision study of nuclear reactions. Some experiments in atomic physics require slower ions; bare ions decelerated and trapped at thermal energy and confined in a small volume would be ideal for these experiments. The beam blow-up inherent to all means of decelerating beams requires cooling at different energies to maintain the small phase-space volume. Electron cooling is very fast and can compensate for such blow-up when an ion beam repeatedly traverses a thin target inside the ring. This combination of electron cooling and internal target is a new technique that has yet to be fully exploited.

When an energetic ion beam hits a nuclear target, unstable nuclei (nuclear fragments) are produced, and those that have lifetimes of several minutes or more can be collected. As in the case of antiproton production, these initially diffuse ion beams have to be cooled so that many pulses can be accumulated to give high beam intensity. But the ions are slower than antiprotons and can be more easily matched in velocity with electrostatically accelerated electrons. Thus electron cooling is here more appropriate than stochastic cooling because it provides shorter cooling times and smaller beams.

The new GSI (Gesellschaft für Schwerionenforschung) accelerator complex[10] is described in Box 1 as an example to illustrate the production and use of fully stripped ions and nuclear fragments. In this instrument, the high quality of the beam and the combination of electron cooling and an internal target permits nuclei and nuclear reactions to be studied in great detail. There is also the possibility of attaching one electron to the bare nucleus and measuring its binding energy. A single-electron heavy ion is preferred to a multielectron ion for testing the predictions of quantum electrodynamics (QED) because there is no residual electron cloud to complicate the analysis of the energy levels.

In some cases the cooling ring can be used directly as a mass spectrometer; this is particularly useful for measuring the mass of unstable nuclei.

Principles of electron cooling

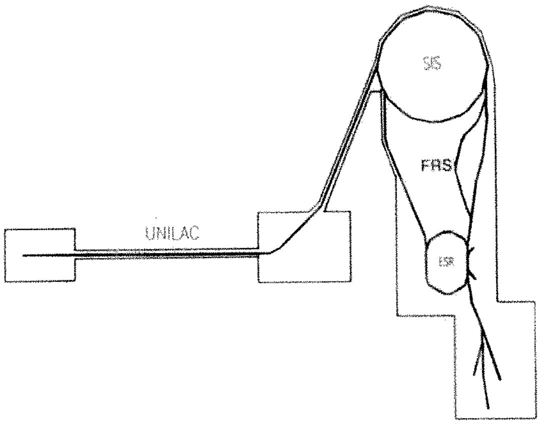
To effect cooling, the ion beam must be overlapped with cold electrons of the same velocity. The electron energy is therefore lower by a factor m_e/m_i (the ratio of the electron mass to the ion mass, 1/1,836 for protons). Consequently, ion beams in the MeV energy range can be cooled by keV electrons. Such electron energies may be attained using well established technologies—they are reached in X-ray tubes, for example.

Electron and ion beams are merged in a straight section of the storage ring (see Box 1). In the moving (orbiter) frame the system appears as a hot ion gas mixed with a cold electron gas. Temperature relaxation takes place through Coulomb collisions between electrons and ions. The ions circulate continuously in the storage ring but the electrons are removed at the end of the overlap region, because they can be delivered constantly and so do not need to be recirculated. The cooling effect appears in the laboratory frame as a reduction in the momentum spread (longitudinal cooling)—being slower than electrons, the ions are accelerated slightly by them (faster ions will accelerate the electrons while being themselves decelerated)—and as a reduction of beam size and divergence (transverse cooling), because ions entering obliquely into the parallel electron beam will receive a kick into the forward direction and their transverse velocity component will be reduced.

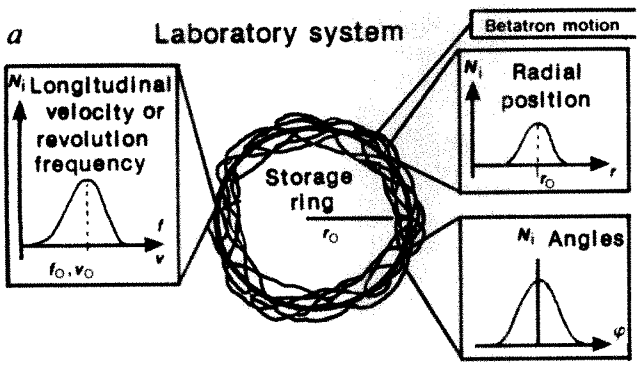
Because the ions move around the storage ring about a million times per second, there is a frequent repetition of these processes in a short interval, providing cooling times of the order of seconds. Ideally, both beams eventually come into thermal equilibrium, which means that the ion-beam temperature is reduced from several million degrees to about 1,300 K in the transverse direction, and to less than 10 K longitudinally. The transverse temperature is identical to that of the electrons when they leave the thermionic cathode; the longitudinal temperature

Box 1 The GSI accelerator complex

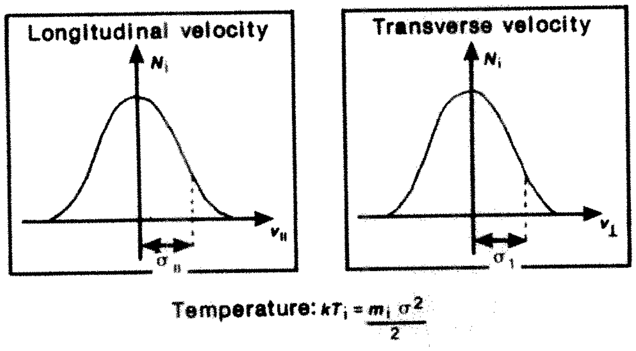
This device illustrates the production of fully stripped ions and nuclear fragments. Partially stripped ions are accelerated in two stages—first in a linear accelerator (UNILAC) and then in a synchrotron (SIS)—to an energy greater than the stripping threshold of the innermost electron. They may then be either passed through a metal foil to produce fully stripped heavy ions or fired at a target to produce a beam of nuclear fragments (FRS). Either species can be injected into a ring (ESR) for electron cooling. The injected beam has a large spread in longitudinal velocity, and is smeared out in the transverse direction because of 'betatron' oscillations (*a*). In the rotating (orbiter) frame, the particle velocities are distributed around zero (*b*) with a r.m.s. width σ that is related directly to the beam temperature. Electron cooling of the ion beam narrows these distributions and thus reduces the beam size, divergence and momentum spread. The cooled beam can then be accelerated or decelerated as required, or used directly in combination with an internal target in the ring.



a Laboratory system



b Accompanying (orbiter) system



is lower, as the longitudinal velocity spread of the electrons, after acceleration, is compressed in the orbiter frame. Thermal equilibrium is reached only under ideal conditions, because the ions scatter from one another frequently when the ion beam becomes very dense, transferring energy from transverse to longitudinal motion; this mechanism acts as a heating process.

We can also consider the electrons as a foil moving with velocity v_0. The cooling process can then be understood as a cumulative energy loss experienced by the ions due to repeated passages through the foil. The energy loss reduces the ion velocity along the direction of motion until it equals v_0, with the velocity vector parallel to that of the electrons.

Theory of electron cooling

Considering the cooling process as arising from the friction of ions passing through an electron 'foil', the corresponding energy loss in the moving frame is given simply by the Bohr formula

$$F(v_i) = \frac{dE}{dx} = -4\pi Z_i^2 n_e r_e^2 m_e c^2 L_{\mathrm{Coul}} \frac{c^2}{v_i^2} = -F_0 \frac{c^2}{v_i^2} \qquad (1)$$

where n_e is the number density of the electrons, Z_i and v_i are the charge and the velocity of the ion, r_e is the classical electron radius (2.8×10^{-15} m), L_{Coul} is the Coulomb logarithm (a dimensionless constant of order 10 which characterizes the scattering process), E is the ion's energy and F is the retarding force. The equation shows that the energy loss scales inversely with the square of the ion velocity; the quantity F_0 subsumes the preceding cluster of variables. In deriving the frictional or cooling force, which is equivalent to the energy loss, one must take into account the non-zero temperature of the electrons. This requires taking an average over the corresponding electron velocity distribution $f(v_e)$

$$F(v_i) = -F_0 c^2 \int f(v_e) \frac{v_i - v_e}{|v_i - v_e|^3} d^3 v_e \approx -\tfrac{3}{2} F_0 c^2 \frac{v_i}{v_i^3 + 2\Delta_e^3} \qquad (2)$$

This removes the divergence at $v_i = 0$ in equation (1) and leads to a drop in the force for ion velocities below the electron velocity spread Δ_e (Fig. 1). The approximation holds for a spherical maxwellian electron-velocity distribution with electron temperature $kT_e = \frac{1}{2} m_e \Delta_e^2$. The friction rate (change in velocity per unit time divided by the velocity) is obtained by dividing the force by the ion momentum. The inverse of this quantity is called the cooling time τ. From equation (2)

$$\tau = -\frac{m_i v_i}{F} = \frac{2}{3} m_i \frac{v_i^3 + 2\Delta_e^3}{F_0 c^2} \qquad (3)$$

Thus the cooling time is proportional to v_i^3 (where the velocity is in the orbiter frame) if $v_i \gg \Delta_e$, and is constant for $v_i \ll \Delta_e$. To obtain the cooling time in the laboratory frame, τ_{lab}, we must multiply by the Lorentz factor γ^2 (where $\gamma = 1/\sqrt{1-\beta^2}$ and $\beta = v_i/c$) and by the ratio of ring circumference C to the length of the cooling section, l. If the ion velocity distribution in the orbiter frame is gaussian, v_i can be replaced by the r.m.s. width σ_i to obtain the temperature relaxation time

$$\tau_{\mathrm{lab}} = \frac{C\gamma^2}{l} \tau \approx \frac{4\sqrt{2}}{3} \frac{m_i c}{F_0} \left[\left(\frac{kT_i}{m_i c^2} \right)^{3/2} + 2 \left(\frac{kT_e}{m_e c^2} \right)^{3/2} \right] \frac{C\gamma^2}{l} \qquad (4)$$

This formula (except for the factor $C\gamma^2/l$) was presented 50 years ago by Spitzer[23,24] for temperature relaxation phenomena in plasma physics. It describes the main features of electron cooling, but neglects important details for the cooling of beams with velocity spreads σ_i smaller than the transverse electron velocity spread Δ_e. It represents a conservative estimate, but describes the basic scaling behaviour of the cooling time, namely its increase as the $\frac{3}{2}$ power of the ion-beam velocity for hot ion beams ($\sigma_i \gg \Delta_e$), its increase with decreasing electron density, and its decrease with ion charge ($\tau_{\mathrm{lab}} \propto m_i/Z_i^2 \approx 1/Z_i$). It can also be seen that at low energies τ_{lab} is nearly constant for constant electron density. At highly relativistic energies, however, τ_{lab} becomes very long because of the γ^2 dependence. For typical parameters ($n_e = 10^8\ \mathrm{cm}^{-3}$, $L_{\mathrm{Coul}} = 10$, $T_e = 1{,}300$ K, $C/l = 25$, $\sigma_i = \Delta_e$, $\gamma = 1$) we obtain $\tau_{\mathrm{lab}} = 1$ s for protons; for fully stripped uranium ions it would be 35 times smaller.

The basic theory of electron cooling[11,12] was developed using a binary collision model. It has been refined by Sørensen and Bonderup[15], who treated the plasma behaviour of the electrons in more rigorous way. To achieve efficient cooling, a cold electron beam is required, the generation of which is described below.

Electron cooling devices

Essential points in the design of an electron cooling device are the achievement of a low electron-beam temperature, operation under ultrahigh-vacuum conditions, adiabatic acceleration of the electrons to the ion-beam velocity, and 'clean' injection of the electrons into the collector. The electron gun must be designed carefully and a highly stable high-voltage power supply is needed. A longitudinal magnetic field is used to guide the electron beam and to prevent it from blowing up through mutual repulsion of the electrons. In the cooling region, local variations of the magnetic-field direction must be smaller than 0.1 mrad. Powerful vacuum pumps close to the gun and the collector keep the pressure in the cooling region at about 10^{-11} torr or less. At the end of the cooler the electron beam must be decelerated carefully, reducing its power from around several hundred to a few kilowatts, to prevent the collector from being damaged or heated. Good collectors usually capture more than 99.9% of all primary electrons. Figure 2 illustrates a typical electron cooler: it shows the apparatus[25,26] used for the cooling of slow antiprotons in the Low-Energy Antiproton Ring (LEAR) at CERN.

Electron cooling experiments

The earliest experiments were concerned with measuring the cooling times and frictional forces under various conditions. The results[5-8,27,28] obtained in different laboratories are remarkably consistent. The time required for reducing the transverse

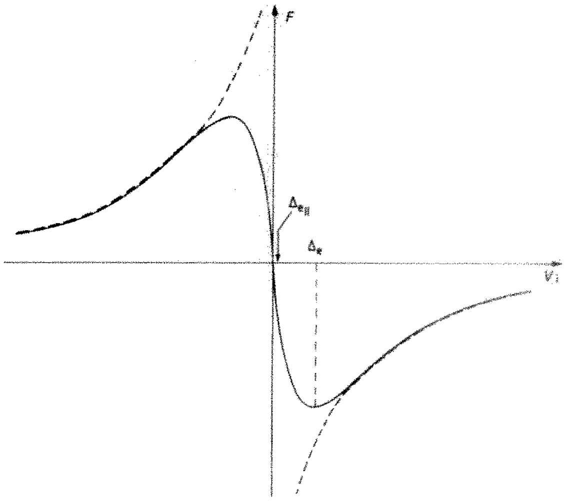


FIG. 1 Cooling force F as a function of the ion velocity in a reference frame moving with the velocity v_0 of the electrons. Dashed line: cooling force for electrons at temperature $T_e = 0$ K; solid line: cooling force for electrons at temperature $T_e = \frac{1}{2} m_e \Delta_e^2$. The arrow shows the maximum of the cooling force for a electron velocity distribution compressed in the longitudinal direction to a spread $\Delta_{e\parallel}$.

extension of a beam was measured as a function of the transverse velocity $v_{i\perp}$ (Fig. 3*a*) and was found to be proportional to $v_{i\perp}^{-2}$. But equation (3) predicts a $v_{i\perp}^{-3}$ dependence for $v_{i\perp} \gg \Delta_e$ and no dependence on $v_{i\perp}$ for $v_{i\perp} \ll \Delta_e$, so this observation that the cooling time decreases continuously with decreasing ion velocity was unexpected. Such '(super)fast' cooling is well described, however, by a more elaborate theory[12,15] and results from the longitudinal magnetic field (solenoid) in which the electrons move. This field transforms an electron's transverse motion into a spiral about the magnetic field lines (cyclotron motion). Scattering of ions by electrons at collision cross-sections larger than the radius of these cyclotron orbits averages out the transverse electron motion, so that only the longitudinal velocity spread $\Delta_{e\parallel}$ is relevant. Thus the position of the maximum in Fig. 2 is shifted from Δ_e to $\Delta_{e\parallel}$, which is usually much smaller, resulting in a considerable increase in the frictional force for small ion velocities in the orbiter frame. The force component associated with this phenomenon is called the magnetic cooling force.

In the transverse direction one usually measures the cooling time, whereas in the longitudinal direction the cooling force can be determined directly. This is achieved by abruptly detuning the electron velocity v_0 by a small amount δ after equilibrium is reached. The ion-beam velocity is then changed to the value $v_0 + \delta$ as a consequence of the cooling force. The time taken by the ion beam to reach the new velocity is directly proportional to the longitudinal cooling force. This is illustrated in Fig. 3*b*.

The effect of the longitudinal force on an uncooled beam is to reduce the momentum spread. Figure 4 shows consecutive scans (at intervals of 1 s) of the momentum distribution of a proton beam immediately after injection. The initial momentum spread of 0.3% is reduced within a few seconds by a factor of about 100. Close inspection of the narrow peak thus produced (inset to Fig. 4) yields another interesting observation[27]: at a very low beam temperature, the ions can no longer be considered as independent. The energy of the particles in the rest frame becomes comparable to the mutual Coulomb force, and collective motions can build up easily. These collective fluctuations can be represented as two waves moving with or against the direction of the beam[29]: one group of particles moves faster and another moves slower than the nominal velocity of the ions, resulting in the two peaks seen in the inset to Fig. 4. It is predicted that the temperature of the particle beam can become so low that the energy of the particle in the rest frame becomes lower than the Coulomb force at a distance corresponding to the average distance between ions[30]. In this case, the ions could no longer pass each other, and would circulate around the ring in a configuration that resembles a crystal, held together by their mutual electrical interactions. Indications of linear ordering have been reported[31,32] whereby the protons move around the ring in a formation analogous to a string of pearls. If it indeed exists, this effect should be stronger, at a given temperature, for highly charged heavy-ion beams, and more complex structures have been predicted[33-36]. The experimental realization of this effect and its unambigous theoretical proof are challenges for the new generation of cooling experiments proposed in heavy-ion storage rings.

TABLE 1 Smallest values of ion-beam parameters obtained by electron cooling

Beam radius	10 μm
Beam divergence	0.1 mrad
Beam emittance	0.1 π mm mrad
Transverse temperature	1,000 K
Momentum spread (r.m.s.)	1×10^{-6}
Longitudinal temperature	1 K
Shortest cooling time	25 ms
Highest cooling force	1 eV m^{-1}
Lowest beam energy (protons)	1.5 MeV
Highest beam energy (protons)	287 MeV
Cooled ions	p, H^-, $\bar{p}$, Li^+, C^{6+}, C^{5+}, O^{8+}, O^{7+}, O^{6+}, Si^{14+}

As can seen from Fig. 3*b*, the friction force can become very strong, and its effect can be seen after only a single passage. The Novosibirsk group has shown that, after a single pass, the cooling force at its maximum is about 2.5 times stronger for negative ions (H^-) than for protons[37]. This is because the trajectories of magnetically bound electrons are different when they scatter from a positive rather than a negative ion. This result has yet to be confirmed by comparing the cooling of protons and antiprotons.

For a long time it was widely held that electron cooling could not be applied to highly charged ions because the cooling electrons would be captured during the cooling process and the 'down-charged' ions that result would leave the storage ring. Electron capture was indeed observed in the initial proton-cooling experiments, yielding a relativistic beam of atomic hydrogen. This beam was used to measure the properties of the proton beam in a non-destructive way. The formation rate of hydrogen was of the order of a few thousand atoms per second, as predicted by theory[5,24,38]; this loss rate is almost negligible. The recombination time τ_{rec} (the time taken to convert a fraction 1/e of the beam to down-charged ions) decreases as $1/Z_i^2$ whereas the cooling rate decreases as A/Z_i^2 (see equation (3)),

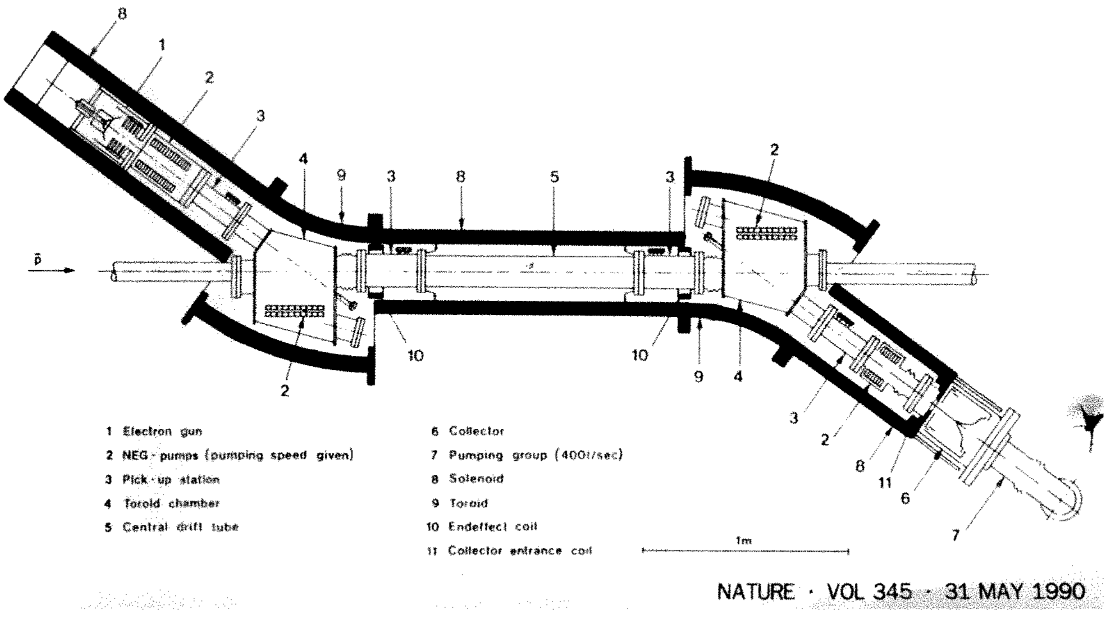


FIG. 2 A typical electron cooling device[25,26]. Electrons are extracted from the hot cathode and accelerated in the gun to the desired velocity. They drift in a longitudinal magnetic field to the cooling section. The merging with and separation from the ion beam are achieved with toroid magnets. After the cooling process, electrons are decelerated and captured in a collector. Vacuum pumps and diagnostic elements are distributed over the whole system.

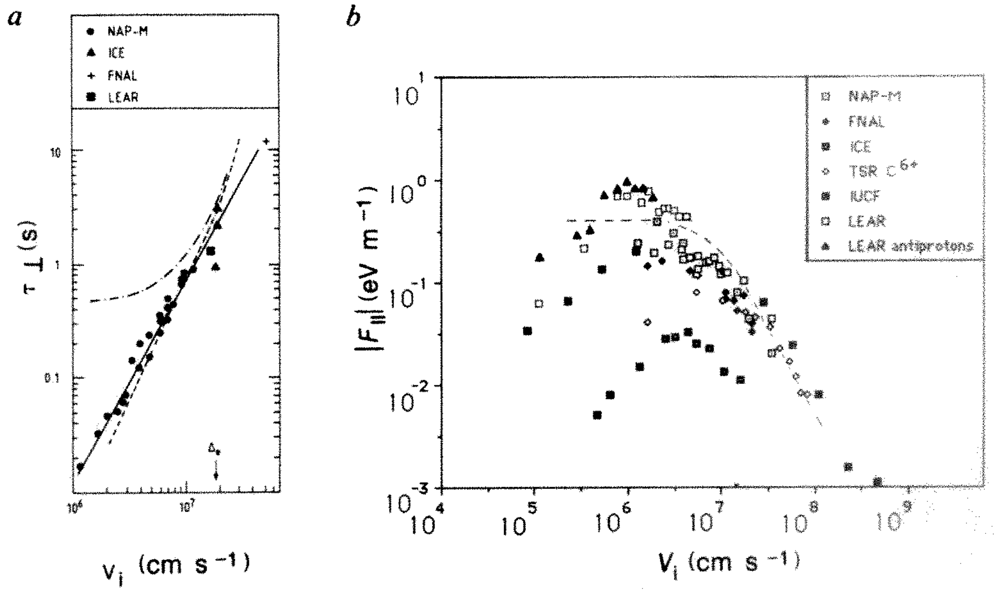


FIG. 3 Summary of electron cooling measurements for several different instruments. *a*, Transverse cooling times as a function of the ion velocity in the moving reference frame. Dashed-dotted line indicates the prediction of equation (3) (normalized to $n_e = 10^8\,\text{cm}^{-3}$ and $C/l = 50$), the dashed and solid lines are fits to the data with a model including the magnetic force. *b*, Longitudinal frictional force as a function of longitudinal ion velocity in the moving frame. The dashed line indicates the contribution from the non-magnetic force.

so the ratio τ_{rec}/τ is proportional to the nuclear mass *A*. For bare uranium ions the cooling time is expected to be at least a hundred times shorter than the recombination time, so that recombination is not significant over many times the cooling period τ.

Electron cooling of a 'stationary beam' of antiprotons has been carried out recently[39]; antiprotons in a Penning ion trap were cooled from a few keV to below 1 eV. Cooling times of the order of 10 s were determined.

Some of the state-of-the-art values from electron cooling experiments[5-9,27,28,40] are summarized in Table 1; for more details, see ref. 21.

Cooling ring projects

The exciting initial results of electron cooling have triggered the development of a number of storage rings for light and heavy ions equipped with coolers[41-49]. These will provide a variety of ions and will operate over a wide range of energy (Fig. 5). The main applications of electron cooling in these instruments are summarized in Box 2. I shall discuss here some important examples.

When antiprotons are scattered from a polarized proton target, they may annihilate more frequently in one spin state than in another. This fact can be used to filter out one spin state and thus to produce a polarized antiproton beam[50]. A pure polarized proton target may be obtained for this purpose by directing a beam of atomic hydrogen through the vacuum chamber of the storage ring. But because such an atomic-beam target is very thin, the antiproton beam has to pass through it many times to become polarized. Therefore the antiprotons that do not annihilate on traversing the atomic beam are recirculated. Electron cooling is needed to counteract multiple (electromagnetic) scattering in the antiproton beam, which can otherwise lead to beam blow-up over many cycles. Gradual depletion of one spin component through annihilation would result ultimately in a polarized antiproton beam circulating in the storage ring. Such a beam has not yet been produced, but would be extremely valuable for the study of the spin dependence of the antinucleon-nucleon force and of the annihilation.

Another application is in pion production. When a projectile collides with a target nucleus, a pion can be produced if the energy available in the centre-of-mass system exceeds a threshold corresponding to the pion rest mass. The production of a charged pion converts a proton into a neutron (π^+ production) or a neutron into a proton (π^- production). The nucleons are internally bound differently, resulting in a production threshold that depends on the final nuclear states[51,52]. A negative pion can become attached to a nuclear fragment created in the same

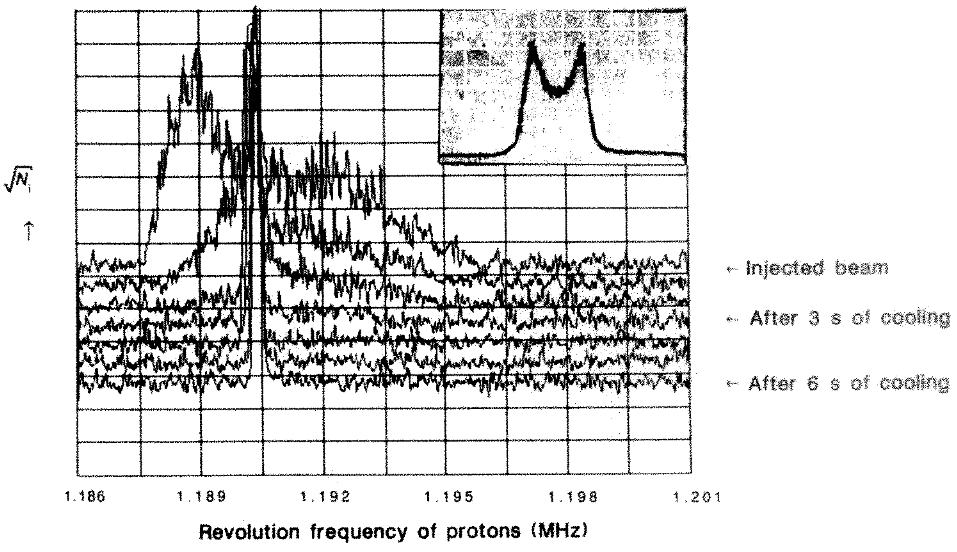


FIG. 4 Longitudinal cooling (cooling of momentum spread) of a stored proton beam in LEAR, showing scans of the momentum distribution immediately after injection of protons at 1-s intervals[27]. The inset shows the final proton signal at the lowest temperature.

Box 2 Application of electron cooling in storage rings

Small beam sizes:	Experiments with high spatial resolution and high luminosity for colliding or merged beams.
Low momentum spread:	High-resolution nuclear and particle spectroscopy.
Long beam lifetime:	Internal-target and storage-ring operation, and beam polarization.
Fast cooling times:	Accumulation of rare ions and unstable isotopes.
High phase-space density:	Efficient deceleration of antiprotons and rare ions for high-resolution atomic spectroscopy.
Low beam temperatures:	Searches for beam ordering and crystallization.

reaction, forming a pionic atom; this introduces additional energy structure (corresponding to the atomic levels) at the threshold energy. To resolve this structure, high energy resolution is needed. The target must be thin to avoid energy smearing of the projectile and of the reaction products. These objectives can be attained using electron cooling and an internal gas target. The thickness of the target is chosen such that multiple scattering blow-up of the ion beam is counteracted by electron cooling. High-resolution energy scans through the threshold region can be performed by incremental changes in the energy of the electron beam, which drags the ion beam accordingly. The energy of the ions can be deduced with high precision from the electron acceleration voltage.

An intriguing possibility is the production of cooled beams of bare heavy ions and unstable nuclei. The short cooling times that can be obtained for highly charged ions allow sufficient quantities to be accumulated for nuclear and atomic spectroscopy. The prospect of producing dense beams of fully stripped heavy ions is appealing also for accelerator physics, because many effects of interest increase with powers of Z_i, so that highly charged ions may provide access to regions that are not easily reached with singly charged ions. Ion-beam crystallization is one such example.

Electrons used for cooling also constitute a very clean target for the study of electron-ion interactions at very low relative energy. The most important reactions of this kind are

$A^{Z+} + e^- \rightarrow A^{(Z-1)+*} + h\nu$	radiative electron capture
$h\nu + A^{Z+} + e^- \rightarrow A^{(Z-1)+*} + h\nu + h\nu$	induced radiative electron capture
$A^{Z+} + e^- \rightarrow (A^{(Z-1)+*} \rightarrow A^{(Z-1)+} + h\nu$	dielectronic recombination
$A^{Z+} + e^- + e^- \rightarrow A^{(Z-1)+*} + e^-$	collisional recombination

where A denotes an ion of mass A and the asterisk refers to an excited atomic state. Processes such as these are important in plasmas and stellar atmospheres. The configuration used in electron cooling (for example, well defined electron energy, no windows, no binding effects) allows their study in heavy-ion storage rings under very clean conditions. The electron beam merged with the cooled ion beam in the interaction region represents a stable two-component plasma that is fully ionized despite its low temperature; this situation is otherwise difficult to produce and maintain. The possibilities for atomic physics are wide-ranging[53]. One important area of investigation is the high-resolution spectroscopy of photons emitted following electron capture. A precise measurement of the electron binding energies in highly charged systems tests several predictions of quantum electrodynamics for large Z_i, a realm that is otherwise scarcely accessible in simple (hydrogen-like) systems. The large single-electron binding energy also permits relativistic effects to be investigated with great sensitivity[53]. The emitted photons could be used as a tunable quasi-monochromatic source of X-rays[54] and the various electron capture processes might be used to extract the ion beam[55].

Antiproton and positron beams could be merged in the same way as electron and proton beams. With such an arrangement it would be possible to produce antihydrogen atoms by recombination[56]. High-resolution atomic spectroscopy of antihydrogen would provide tests of the CPT theorem (the invariance of a system to simultaneous inversion of charge (C), parity (P) and time (T)) by comparing the hyperfine splitting of the antihydrogen ground state with that of hydrogen. The latter has been measured using a hydrogen maser to a precision of 6×10^{-13}. Such precision is not yet possible for antihydrogen, but even a precision of 10^{-6} would yield valuable information about the magnetic moment and the form factor of the antiproton.

There are many other appealing prospects for the study of atomic antimatter[56]. High-resolution experiments with keV antiprotons should be feasible, permitting studies of their scattering from nucleons and their annihilation, as well as their capture into bound states. At present there exist virtually no experimental data for such processes at energies below ~10 MeV. Electron cooling would also permit large numbers of antiprotons to be brought simultaneously to rest and to be captured in a trap.

Discussion

Electron cooling is well tested and is generally well understood. It may now be used to explore new physics, particularly in experiments with non-relativistic or semi-relativistic ions and in the study of extreme states of plasmas.

Experiments using light- and heavy-ion beams profit immediately from electron cooling through phase-space compression, higher reaction rates, and better energy and spatial resolution. Subsecond cooling times, obtainable simultaneously for transverse and longitudinal degrees of freedom, and the attainment of very small beam sizes and momentum spreads, make possible the spectroscopy of nuclear and atomic states with high precision. The combination of electron cooling and use of interna targets will provide a powerful tool for such experiments. The prospects are promising for the deceleration of bare heavy ions to low energy, allowing them to be caught in a trap for the execution of high-resolution mass measurements or atomic spectroscopy. The same is true for radioactive nuclei and the study of their decay.

This is all to say that electron cooling could help to advance the frontiers of precision measurements in atomic, nuclear and low-energy particle physics. Electron cooling may also benefi high-energy physics. The rapid electron cooling of heavy ions after the last stripping stage could be used for the accumulation of many injector pulses in the acceleration cycle of a high-energy

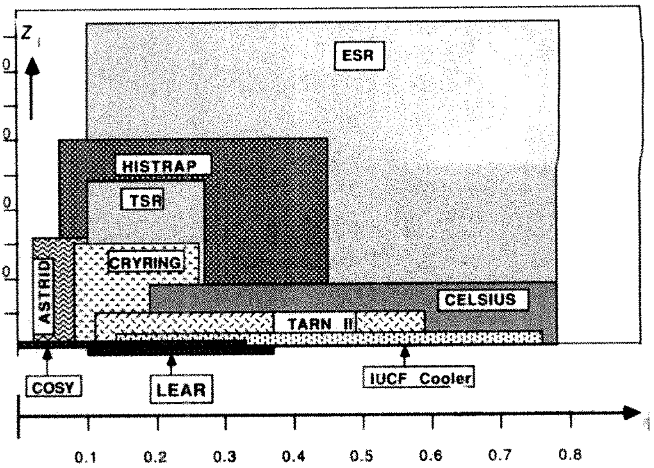


FIG. 5 Range of operation of electron cooling in storage rings; LEAR[25,26] IUCF[40,41], TSR[42], CELSIUS[43], ASTRID[44], ESR[45], TARN II[46], CRYRING[47], COSY[48] HISTRAP[49]. Z_i is the charge on the cooled ions, and β is the electron velocit relative to the speed of light, v_e/c.

heavy-ion accelerator. Accumulation between the cycles of a small ring used to feed a large, high-energy machine could greatly increase the beam luminosity for high-energy experiments. ☐

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1. Budker, G. I. *Proc. Int. Symp. on Electron and Positron Storage Rings* (eds Zyngier, H. & Cremieux-Alcan, E.) III-1 (Presses Univ. de France, Paris, 1967).
2. Van der Meer, S. *CERN/ISR-PO/72-31* (CERN, Geneva, 1972).
3. Möhl, D., Petrucci, G., Thorndahl, L. & Van der Meer, S. *Phys. Rep.* **58,** 73–119 (1980).
4. Van der Meer, S. *Rev. mod. Phys.* **57**, 689–697 (1985).
5. Budker, G. I. *et al. Partical Accelerators* **7,** 197–211 (1976).
6. Bell, M., Chaney, J., Herr, H., Krienen, F. & Petrucci, G. *Nucl. Instrum. Meth.* **190**, 237–255 (1981).
7. Ellison, T. *et al. IEEE Trans. Nucl. Sci.* NS-30, 2636–2638 (1983).
8. Poth, H. *et al. Nucl. Instrum. Meth.* **A287,** 328–332 (1989).
9. Steck, M. *et al Nucl. Instrum. Meth.* **A287,** 324–327 (1989).
10. Blasche, K. & Böhne, D. *Proc. 14th Int. Conf. High Energy Accelerators* Chicago (American Institute of Physics, New York, 1989).
11. Derbenev, Ya. & Skrinsky, A. N. *Particle Accelerators* **8,** 1–20 (1977).
12. Derbenev, Ya & Skrinsky, A. N. *Particle Accelerators* **8,** 235–243 (1978).
13. Ogino, T. & Ruggiero, A. G. *Particle Accelerators* **10,** 197–205 (1980).
14. Bell, J. S. & Bell, M. *Particle Accelerators* **11,** 233–238 (1981).
15. Sørensen, A. & Bonderup, E. *Nucl. Instrum. Meth.* **215,** 27–54 (1983).
16. Budker, G. I. & Skrinsky, A. N. *Soviet Phys. Usp.* **21,** 277–296 (1978).
17. Derbenev, Ya & Skrinsky, A. N. *Phys. Rev.* Vol. 3 (ed. Khalatnikov, I. M.) 165 (Harwood Academic, Chur, 1981).
18. Cole, F. T. & Mills, F. E. *A. Rev. Nucl. Particle Sci.* **31,** 295–325 (1981).
19. Skrinsky, A. N. & Parkhomchuk, V. V. *Soviet J. Particle Nucl.* **12,** 223–247 (1981).
20. Poth, H. *Proc. CERN Accelerator School, CERN* 87-03, 534–569 (CERN, Geneva, 1987).
21. Poth, H. *Phys. Rep.* (in the press).
22. Poth, H. (ed.) *Proc. Workshop on Electron Cooling and Related Applications* (ECOOL84) (KfK 3846, Karlsruhe, 1985).
23. Spitzer, L. *Mon. Not. R. astr. Soc.* **100,** 396–405 (1940).
24. Spitzer, L. *Physics of Fully Ionized Gases* (Interscience Publishers, New York, 1956).
25. Habfast, C. *et al. Phys. Scripta* **T22,** 277–281 (1988).
26. Poth, H. *et al. Proc. 4th Workshop on Physics at LEAR with Cooled Low Energy Antiprotons* (eds Amsler, C. *et al.*) 121 (Harwood Academic, Chur, 1988).
27. Poth, H. *et al. Z. Phys.* **A332,** 171–188 (1989).
28. Wolf, A. *et al. Proc. 1st European Accelerator Physics Conf.* (ed. Tazzari, S.) 205 (World Scientific, Singapore, 1989).
29. Parkhomchuk, V. V. & Pestrikov, D. V. *Soviet Phys. Tech. Phys.* **25,** 818–822 (1980).
30. Dikansky, N. S. & Pestrikov, D. V. *Proc. workshop on Electron Cooling and Related Applications* (ECOOL 84) 275 (KfK 2846, Karlsruhe, 1985).
31. Dementev, E. N., Dikansky, N. S., Medvedko, A. S., Parkhomchuk, V. V. & Pestrikov, D. V. *Soviet Phys. Tech. Phys.* **25,** 1001–1003 (1980).
32. Parkhomchuk, V. V. *Proc. Workshop on Electron Cooling and Related Applications* (ECOOL 84) 71 (KfK 3846, Karlsruhe, 1985).
33. Schiffer, J. P. & Kienle, P. *Z. Phys.* **A321,** 181 (1985).
34. Rahman, A. & Schiffer, J. P. *Phys. Rev. Lett.* **57,** 1133–1136 (1986).
35. Habs, D. *Frontiers of Particle Beams,* (eds Month, M. & Turner, S.) 310 (Springer, Berlin, 1986).
36. Hasse, R. W., Hofmann, I. & Liesen, D. (eds) *Proc. Workshop on Crystalline Ion Beams* (GSI-Report GSI-89-10) (GSI, Darmstadt, 1989).
37. Dikansky, M. S. *et al. Proc. 1st European Accelerator Physics Conf.* (ed. Tazzari, S.) 529 (World Scientific, Singapore, 1989).
38. Bell, M. & Bell, J. S. *Particle Accelerators* **12,** 49–52 (1982).
39. Gabrielse, G. *et al. Phys. Rev. Lett.* **63,** 1360–1363 (1989).
40. Friesel, D. L. *et al. Nucl. Instrum. Meth.* **B40/41,** 927–933 (1989).
41. Pollock, R. E. *IEEE Trans. Nucl. Sci.* **NS-30,** 2056–2060 (1983).
42. Jaeschke, E. *et al. Proc. 1st European Accelerator Physics Conf.* (ed. Tazzari, S.) 365 (World Scientific, Singapore, 1989).
43. Eckström, C. *et al. Phys. Scripta* **T22,** 256–268 (1988).
44. Stensgard, R. *Phys. Scripta* **T22,** 315–317 (1988).
45. Franzke, B. *Nucl. Instrum. Meth.* **B24/25,** 18–25 (1987).
46. Noda, A. *et al. IEEE Trans. Nucl. Sci.* **NS-32,** 2684–2688 (1985).
47. Herrlander, C. J., Bárány, A., Rensfelt, K.-G. & Starker, J. *Phys. Scripta* **T22,** 282–285 (1988).
48. Pfister, U. *Proc. 1st European Accelerator Physics Conf.* (ed. Tazzari, S.) 398 (World Scientific, Singapore, 1989).
49. Olsen, D. K. *et al. Nucl. Instrum. Meth.* **B24/25,** 26–37 (1987).
50. Steffens, E. *et al. Proc. 3rd LEAR Workshop on Physics with Cooled Low-Energy Antiprotons in the ACOL Era* (eds Gastaldi, U., Klapisch, R., Richard, J.-M. & Tran Thanh Van, J.) 245 (Editions Frontieres, Gif-sur-Yvette, 1984).
51. Ericson, T. E. O. *Proc. Workshop on Nuclear Physics with Stored, Cooled Beams* (eds Schwandt, P. & Meyer, H. O.) 172 (American Institute of Physics, New York, 1985).
52. Kilian, K. *Proc. Workshop on Nuclear Physics with Stored, Cooled Beams* (eds Schwandt, P. & Meyer, H. O.) 319 (American Institute of Physics, New York, 1985).
53. Datz, S., Andersen, L. H., Briand, J.-P. & Liesen, D. *Phys. Scripta* **T22,** 224–227 (1988).
54. Poth, H. & Wolf, A. *Phys. Lett.* **A94,** 135–138 (1983).
55. Poth, H. *Z. Phys.* **A326,** 483–486 (1987).
56. Poth, H. & Wolf, A. (eds) *Proc. Symp. on the Production and Investigation of Atomic Antimatter* (ANTIMATTER '87) (Scientific Publishing Company, Basel, 1988).

ARTICLES

Calibration of the ^{14}C timescale over the past 30,000 years using mass spectrometric U–Th ages from Barbados corals

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Uranium–thorium ages obtained by mass spectrometry from corals raised off the island of Barbados confirm the high precision of this technique over at least the past 30,000 years. Comparison of the U–Th ages with ^{14}C ages obtained on the Holocene samples shows that the U–Th ages are accurate, because they accord with the dendrochronological calibration. Before 9,000 yr BP the ^{14}C ages are systematically younger than the U–Th ages, with a maximum difference of ~3,500 yr at ~20,000 yr BP. The U–Th technique thus provides a way of calibrating the radiocarbon timescale beyond the range of dendrochronological calibration.

RECENTLY a suite of coral cores has been raised off the island of Barbados, and more than 30 *Acropora palmata* samples were dated by conventional ^{14}C techniques[1]. The analyses showed that the last deglaciation started at ~15,000 ^{14}C yr BP (before present), and that it occurred in two steps—observations in full agreement with ^{18}O records in deep-sea cores dated by accelerator mass spectrometry (AMS) of ^{14}C (refs 2-4). The Barbados offshore collection of corals is also ideally suited for addressing two important problems: the timing of the last glacial cycle and the calibration of the ^{14}C timescale back to 30 kyr BP.

It is now well documented that radiocarbon dating is not a totally accurate chronometer because the atmospheric ^{14}C/C reference level has changed with time. The first evidence of a discrepancy between true age and ^{14}C age, Δ^{14}C, was reported by De Vries[5], and the most accurate evidence of changes of the ^{14}C/C ratio in the atmosphere (expressed as ‰ above a modern standard, ref. 6) comes from the dating of tree rings[7]. Over the past 9,000 yr, the Δ^{14}C curve is characterized by a long decreasing trend with a maximum difference of ~100‰ at about 7,000 yr BP. This trend has been correlated with changes of the magnetic dipole which acts as a shield to the production of ^{14}C by the cosmic rays. Whether or not this slow change is the result of variations in the ^{14}C-reservoir exchange rate rather than changes of the cosmogenic production is still being debated[8-11]. Superimposed on the long trend are oscillations[12-14], which are attributed to short-term variations of the magnetic properties of the solar wind[13,14]. Before 9,000 yr BP the ^{14}C timescale is not accurately calibrated because of the lack of suitable fossil trees for dendrochronological dating. Several authors have attempted to extend the calibration using other means of establishing absolute ages: varved sediment[15,16], U-Th[17-19] and ice cores[20]. The results obtained are not very precise and are often in disagreement with one another (up to 1,000‰). Nevertheless it seems that before 9,000 yr BP the ^{14}C ages are consistently younger than the true ages, suggesting higher $^{14}C/C$ ratios than present (a positive $\Delta^{14}C$).

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Recently ^{234}U-^{230}Th dating of corals by thermal ionization mass spectrometry (TIMS) has been demonstrated[21-25] to be significantly more precise and accurate than the α-counting method. It has also been predicted that U-Th dating would be even more precise than the ^{14}C in the time range of the last deglaciation (6-20 kyr). We have implemented this technique at the Lamont-Doherty Geological Observatory and measured Th and ^{14}C ages in the same coral samples to enable comparison of the two geochronometers. Here we focus on the U-Th/^{14}C comparison for the past 30,000 years. The implications of the data for the timing of the last glacial cycle as well as other U-Th ages obtained on older corals (back to 130,000 yr) will be described elsewhere (E.B., B.H. and R.G.F., manuscript in preparation).

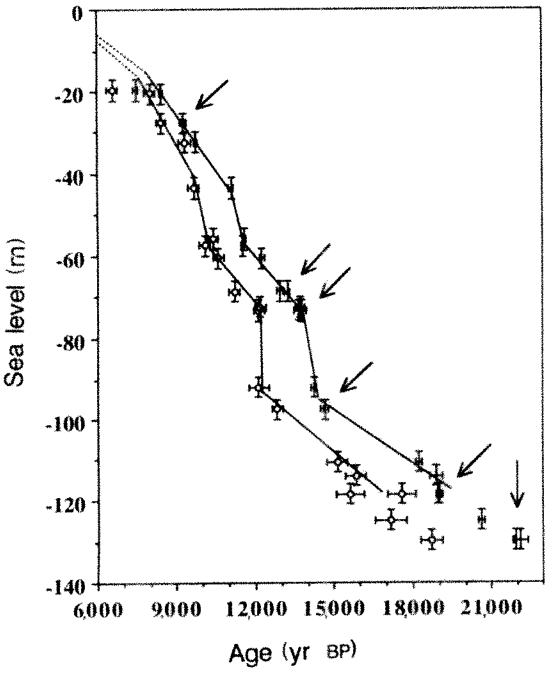


FIG. 1 Sea level during the last deglaciation. Crosses correspond to U-Th ages and the open symbols to ^{14}C ages (calculated with the 5,730-yr half-life). Highest and two lowest samples are composed of *Porites asteroides* and thus only represent lower bound for the sea level at those times. All other samples are *Acropora palmata*. The age errors are quoted at 2σ. The samples marked by arrows correspond to duplicate analyses of the same coral sample by U-Th mass spectrometry. All duplicates are in agreement within the limits of error. A linear uplift correction has been applied by assuming that the last interglacial highstand was at 7 m above the present sea level. This correction is of the order of the inherent error due to *A. palmata* habitat range (±2.5 m).

Methods

All isotope measurements were performed on a VG Micromass 30, single-collector mass spectrometer, equipped with an analog Daly detector for the analysis of small-abundance isotopes. The techniques used for chemical separation and mass spectrometry of U and Th have been adapted from ref. 22. They will be described in detail elsewhere (E.B., B.H., R.G.F. and A.Z., manuscript in preparation).

For thorium we used a ^{229}Th spike for measurement of ^{230}Th by isotope dilution. The absolute concentration of this spike was calibrated against a dilution of a SPEX ThO_2 standard. For the corals measured so far, the $^{232}Th/^{230}Th$ ratios are generally lower than 30 and therefore no correction for detrital ^{230}Th is required because the typical crustal $^{232}Th/^{230}Th$ values are of the order of 10^5 (ref. 23). For uranium we used a ^{236}U spike with 99% enrichment for the measurement of ^{238}U and ^{234}U abundances. The absolute concentration of this spike was calibrated against a dilution of NBL CRM111 ^{233}U standard. The accuracy of our correction procedures for isotope fractionation and mass 238 contribution was checked using the NBS U-010 solution: for the $^{234}U/^{238}U$ atomic ratio the weighted mean of six measurements is $(5.43 \pm 0.02) \times 10^{-5}$ in agreement with the NBS-certified isotope ratio of $(5.466 \pm 0.06) \times 10^{-5}$.

Results

Table 1 gives the list of U-Th results; as an initial check of the accuracy and precision of our dating technique we dated samples collected from the uplifted reefs of Barbados and Haiti, which correspond to the last interglacial period (samples AFM3, C4 and C1 cf Table 1). These measurements agree with previous α-counting results[26] and confirm that Th ages measured in samples from the last interglacial highstand are restricted to the time between 120 and 130 kyr BP (ref. 23).

The precision of the U-Th dating is typically better than 100 yr (at 2σ) for ages between 6 and 20 kyr, significantly better than that achieved for most ^{14}C dating. In addition, replicate analyses of the same hand samples demonstrate that the method is reproducible within the 2σ errors (nine duplicate couples; see Table 1). Initial $^{234}U/^{238}U$ ratios calculated using the Th ages fall in the range of modern sea water (1.14-1.15; ref. 21) for all deglaciation samples. This shows that their U-Th systematics have not been strongly altered after deposition.

Figure 1 focuses on the coral record of the last deglaciation dated by U-Th and ^{14}C. The implication of this plot on the chronology and structure of the last deglaciation is described elsewhere (E.B., B.H. and R.G.F., manuscript in preparation). It is remarkable that there is not even a single stratigraphic inversion in the U-Th data. This shows that erosion and redeposition of coral fragments are minor problems in our Barbados core collection.

Samples RGF9-27-5 (dated at Geochron) and RGF9-27-4 (dated at Lamont-Doherty) correspond to the same depth in core 9 (5 cm from each other) but the difference in ^{14}C age (1,900 yr) is very large. The ^{14}C date at 17 kyr BP is considered to be anomalous as it produces a stratigraphic inversion in the core-9 sequence (see Table 1 and ref. 1).

The measurements of ^{14}C in corals cannot be used directly for calibration against absolute chronometers because the surface layer of the ocean is not in equilibrium with the atmosphere instead, a steady-state balance is maintained between the input of ^{14}C from the atmosphere and its removal by advection and radiodecay in the water column. The ^{14}C ages obtained on corals must therefore be corrected for the age difference between the atmosphere and the surface ocean. This parameter depends mainly on the upwelling and mixing with old sub-surface water masses. For the low-latitude range the reservoir age is generally ~400 yr (50‰) and is not likely to have changed by more than 100-200 yr during the late Pleistocene[27]. We have therefore adjusted the raw ^{14}C ages by 400 yr in Fig. 1 and the following discussion.

TABLE 1 U/Th and ^{14}C results from Barbados corals

Sample	Species	Depth (m)	^{238}U (p.p.m.)	^{230}Th/^{234}U	^{234}U/^{238}U	(^{234}U/^{238}U)$_{init}$	Th age (yr BP)	^{14}C age (yr BP)	^{14}C$_{5,730}$ (yr BP)	^{14}C lab
RGF7-4-2	Pa	−18	2.65 ± 0.02	0.0667 ± 0.0007	1.1414 ± 0.0065	1.1444 ± 0.0067	7,460 ± 80	6,400 ± 200	6,586	L-DGO
RGF7-5-5	Ap	−19	3.24 ± 0.02	0.0752 ± 0.0004	1.1468 ± 0.0052	1.1504 ± 0.0053	8,450 ± 50	7,780 ± 220	8,006	Geochron
RGF7-12-2#1	Ap	−25	3.16 ± 0.02	0.0823 ± 0.0008	1.1359 ± 0.0079	1.1395 ± 0.0081	9,280 ± 100	8,200 ± 200	8,439	L-DGO
RGF7-12-2#2			3.31 ± 0.02	0.0820 ± 0.0007	1.1455 ± 0.0088	1.1494 ± 0.0091	9,250 ± 80			
RGF7-16-2	Ap	−30	3.19 ± 0.01	0.0861 ± 0.0004	1.1456 ± 0.0040	1.1497 ± 0.0041	9,730 ± 50	9,050 ± 250	9,313	Geochron
RGF7-27-4	Ap	−41	3.45 ± 0.02	0.0975 ± 0.0006	1.1411 ± 0.0061	1.1456 ± 0.0063	11,090 ± 70	9,400 ± 200	9,673	L-DGO
RGF12-5-2	Ap	−53	3.17 ± 0.02	0.1016 ± 0.0005	1.1407 ± 0.0043	1.1454 ± 0.0045	11,590 ± 60	10,100 ± 200	10,394	L-DGO
RGF12-6-7	Ap	−55	3.04 ± 0.02	0.1011 ± 0.0006	1.1463 ± 0.0062	1.1512 ± 0.0064	11,530 ± 70	9,800 ± 200	10,085	L-DGO
RGF12-9-5	Ap	−58	3.04 ± 0.01	0.1072 ± 0.0007	1.1462 ± 0.0056	1.1514 ± 0.0058	12,260 ± 90	10,300 ± 200	10,600	L-DGO
RGF12/16/05#1	Ap	−65	2.96 ± 0.01	0.1151 ± 0.0009	1.1399 ± 0.0048	1.1453 ± 0.0050	13,220 ± 110	10,900 ± 200	11,217	L-DGO
RGF12/16/05#2			3.02 ± 0.01	0.1130 ± 0.0010	1.1402 ± 0.0071	1.1455 ± 0.0074	12,970 ± 120			
RGF12-21-6	Ap	−69	3.03 ± 0.02	0.1190 ± 0.0014	1.1446 ± 0.0064	1.1503 ± 0.0067	13,700 ± 170	11,850 ± 200	12,195	L-DGO
RGF12/21/10#1	Ap	−70	3.33 ± 0.02	0.1198 ± 0.0011	1.1335 ± 0.0057	1.1388 ± 0.0060	13,800 ± 140	11,800 ± 200	12,143	L-DGO
RGF12/21/10#2			3.21 ± 0.02	0.1186 ± 0.0011	1.1395 ± 0.0107	1.1450 ± 0.0112	13,660 ± 140			
RGF9-8-2	Ap	−89	3.06 ± 0.02	0.1233 ± 0.0008	1.1372 ± 0.0069	1.1429 ± 0.0072	14,230 ± 100	11,800 ± 400	12,143	L-DGO
RGF9-13-3#1	Ap	−94	3.22 ± 0.02	0.1267 ± 0.0013	1.1309 ± 0.0058	1.1365 ± 0.0061	14,660 ± 160	12,500 ± 200	12,864	L-DGO
RGF9-13-3#2			3.22 ± 0.02	0.1271 ± 0.0008	1.1314 ± 0.0061	1.1370 ± 0.0064	14,700 ± 100			
RGF9-21-11	Ap	−106	3.52 ± 0.03	0.1552 ± 0.0011	1.1358 ± 0.0039	1.1430 ± 0.0042	18,240 ± 140	14,700 ± 400	15,128	L-DGO
RGF9/24/04	Ap	−109	3.36 ± 0.02	0.1603 ± 0.0019	1.1346 ± 0.0079	1.1420 ± 0.0084	18,890 ± 250	15,400 ± 400	15,848	L-DGO
RGF9-27-5#1	Ap	−114	3.43 ± 0.01	0.1610 ± 0.0007	1.1363 ± 0.0050	1.1439 ± 0.0053	18,980 ± 90	17,085 ± 520	17,582	Geochron
RGF9-27-5#2			3.45 ± 0.02	0.1614 ± 0.0008	1.1391 ± 0.0055	1.1468 ± 0.0058	19,030 ± 100			
RGF9-27-4	Ap	−114						15,200 ± 400	15,642	L-DGO
RGF9-32-4	Pa	−120	3.15 ± 0.02	0.1735 ± 0.0009	1.1329 ± 0.0048	1.1409 ± 0.0051	20,610 ± 120	16,700 ± 600	17,186	L-DGO
RGF9-34-8#1	Pa	−124	3.22 ± 0.01	0.1836 ± 0.0011	1.1335 ± 0.0041	1.1421 ± 0.0044	21,930 ± 150	18,200 ± 400	18,730	L-DGO
RGF9-34-8#2			3.16 ± 0.02	0.1851 ± 0.0019	1.1349 ± 0.0094	1.1437 ± 0.0101	22,130 ± 260			
RGF12-30-2#1	Ap	−78	3.22 ± 0.02	0.2458 ± 0.0016	1.1255 ± 0.0067	1.1368 ± 0.0074	30,470 ± 240	27,120 ± 1,520	27,909	Geochron
RGF12-30-2#2			3.46 ± 0.02	0.2428 ± 0.0014	1.1314 ± 0.0062	1.1431 ± 0.0068	30,040 ± 210			
AFM3#1	Ap	55	3.04 ± 0.02	0.6944 ± 0.0046	1.1077 ± 0.0072	1.1536 ± 0.0110	125,100 ± 1,700			
AFM3#2			3.12 ± 0.01	0.6941 ± 0.0026	1.1065 ± 0.0037	1.1518 ± 0.0057	125,100 ± 1,000			
Haiti C4#1	Ap	52	2.95 ± 0.01	0.6897 ± 0.0043	1.1094 ± 0.0072	1.1553 ± 0.0109	123,600 ± 1,600			
C4#2			2.95 ± 0.01	0.6937 ± 0.0035	1.1071 ± 0.0051	1.1526 ± 0.0078	124,900 ± 1,300			
Haiti C1	Ap	52	3.10 ± 0.01	0.6843 ± 0.0030	1.1070 ± 0.0050	1.1512 ± 0.0075	121,900 ± 1,100			

Depth or altitude of recovery are relative to the present sea level. Ap and Pa refer respectively to *Acropora palmata* and *Porites asteroides* samples. The measured atomic ratios have been converted to activity ratios by using the same U–Th half-lives used in ref. 22 (all errors are given by 2σ). Labels #1 and #2 correspond to duplicate U/Th analyses. The initial ^{234}U/^{238}U ratios are obtained by using the Th ages to correct the measured isotope ratios. L–DGO is the Lamont–Doherty Geological Observatory. Note that there is no significant change of uranium concentration between glacial and Holocene samples. The ^{14}C ages correspond to conventional ages (calculated with the 5,568-yr half-life) and ages calculated with the true 5,730-yr half-life. The ^{14}C ages have been corrected for fractionation and a reservoir age of 400 yr (ref. 27).

The most striking result is that the Th ages are consistently older than the ^{14}C ages, with a maximum difference of ~3.5 kyr at ~20 Th kyr BP (Fig. 2). The difference between the two chronometers is equivalent to a Δ^{14}C of ~400–500‰ above the present-day reference level for ages between 18 and 22 kyr BP.

Comparison with previous ^{14}C calibration

The three youngest ^{14}C-dated coral samples (RGF7-4-2, RGF7-5-5 and RGF7-12-2, Table 1) from the Barbados collection are in the range of the precise tree-ring calibration. Our U–Th ages for these samples are in agreement with the dendrochronological ages (7,440–7,140, 8,970–8,380 and 9,420–8,970 yr BP are the calibrated ^{14}C dates to be compared with the Th ages of samples RGF7-4-2, RGF7-5-5 and RGF7-12-2, respectively). For the comparison we used the mixed-layer calibration curve of Stuiver *et al.*[28] and for the oldest sample, RGF7-12-2, we used unpublished data (M. Stuiver, personal communication). The good agreement between the two independent calibrations is a confirmation of the accuracy of the U–Th chronometer in corals, at least for the Holocene (see refs 22, 24 for the accuracy on very young corals). Before the Holocene, we cannot yet demonstrate that our U–Th ages are true 'calendar' ages, but we will argue below that this is a reasonable assumption.

Based on a comparison between varve counts and ^{14}C dating in Lake of the Clouds[15], the calibration curve was extended to 11 kyr BP. There is also good agreement between the varve estimates and the U–Th/^{14}C comparison (Fig. 2).

From 9 to 13 kyr BP, attempts have been made to use the Swedish varve chronology to extend the ^{14}C calibration. These sediments were never directly dated by ^{14}C, however, and the Swedish chronology was tied to the present only recently[29]. Even in its latest version the Δ^{14}C curve based on the Swedish varves correlation is still not in agreement with the Lake of the Clouds record for the interval between 8 and 11 kyr BP and also disagrees with our Barbados results. Stuiver[30] concluded that a few hundreds of varves are missing in the Swedish chronology.

At ~10,000 ^{14}C yr BP (Preboreal/Younger Dryas boundary) the U–Th calibration (~11,500 Th yr BP) is in relatively good agreement with the dendrochronological estimates (≳11,300 dendro yr BP, ref. 31). These two independent estimates are in disagreement with the correlation between climate spectra dated by annual counting of seasonal variations in the ^{18}O content, dust and acidity curves from the Dye III and Camp Century ice cores (~10,700 yr BP, ref. 20).

A detailed analysis of the U–Th/^{14}C calibration indicates that the true duration of the Younger Dryas climate event (10,000–11,000 ^{14}C yr BP) may have been as long as 1,700 years, in agreement with the qualitative estimates by Oeschger *et al.*[32]. Moreover, it is not inconsistent with the plateau in ^{14}C ages at ~9,500 ^{14}C yr BP seen in the dendrochronological data of ref. 31.

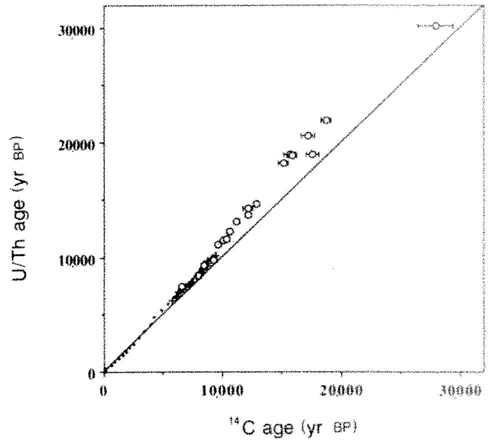


FIG. 2 Open symbols represent the U–Th ages plotted against ^{14}C ages (calculated with the 5,730-yr half-life). The small crosses correspond to the dendrochronological calibration[7] and the large crosses to the Lake of the Cloud varved sediments[15]. For clarity the duplicate analyses by U–Th mass spectrometry have been averaged. The age errors are quoted at 2σ (in the case of U–Th ages they are always smaller than the size of the symbol). For sample RGF9-27-5 we have used the ^{14}C analyses of sample RGF 9-27-5 (Geochron) and RGF9-27-4 (Lamont–Doherty). This study suggests that the ^{14}C age of ~17 kyr is anomalous (stratigraphic inversion, see Table 1). For the three younger samples, the good agreement between the tree-ring calibration and our U–Th/^{14}C estimates indicate that the Th ages are accurate.

Other attempts of radiocarbon calibration have been made by comparing ^{14}C ages with U-Th ages obtained in samples from dried lakes or speleothems[17-19]. For these works the U-Th ages were obtained by α-counting with a much lower precision than by mass spectrometry (the 2σ errors are typically 10-20% of the ages) and a considerable uncertainty is associated with a ^{14}C correction for the initial age of the bicarbonate. Nevertheless, the Barbados U-Th data agree with the study on a south African speleothem[19] and with the Searles Lake record[17,18] if we use the ^{14}C reservoir age for this lake suggested by Broecker and Kaufman[33].

For the 12,000-25,000 ^{14}C yr BP period other authors have found large discrepancies between ^{14}C and K/Ar dating[52] and between ^{14}C and thermoluminescence dating[53], in agreement with our Barbados results.

Possible geochemical artefacts

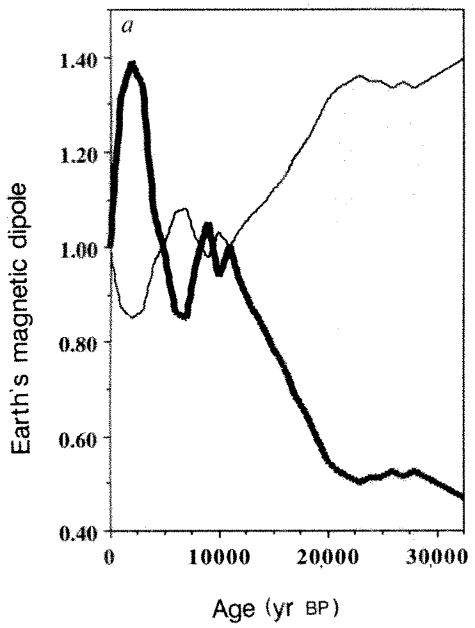
One explanation for the increasing lag between ^{14}C and Th ages could be that the samples were contaminated by recent carbon, either *in situ* and/or during the ^{14}C determinations. To correct the ^{14}C ages back to the Th ages, a contamination of ~4% modern carbon is required. We consider that this is highly unlikely because: (1) replicates conducted so far by AMS do not show any significant difference whether or not the coral sample is strongly leached before processing (conducted on samples RGF9-21-11, RGF12-30-2, RGF1-17-4; E.B., M. Arnold, B. H. and R.G.F., manuscript in preparation); (2) the ^{14}C ages obtained by Fairbanks[1] were measured by β-counting in two different laboratories (Lamont-Doherty, without leaching the samples; Geochron lab, with leaching of the samples); and (3) 2% of contamination is the maximum acceptable to keep the three recent ages in agreement with the tree-ring ages. A definititive test of the accuracy of the ^{14}C ages used in this comparison will be to replicate all the measurements by AMS.

The good quality of the corals for U-Th was tested by X-ray diffraction which showed that no secondary calcite was detectable in the corals. This test is important, but not sufficient, to rule out any diagenetic alteration or uranium exchange[34]. The strongest argument for the quality of the samples comes from the U-Th measurements themselves: all the initial $^{234}U/^{238}U$ ratios fall in the range of the modern sea water (activity ratio between 1.14 and 1.15, ref. 21) in contrast with older air-exposed samples (Table 1, see also ref. 22 and B.H., E.B. and R.G.F., manuscript in preparation).

A gradual loss of uranium could also explain the discrepancy between the two geochronometers. This dissolution can be modelled with first-order kinetics[34]. A rate of uranium loss equivalent to $k = 7\lambda_{234}$ (where λ_{234} is the decay constant of ^{234}U) would be required to reconcile the ^{14}C ages with the U-Th ages. This explanation seems unlikely, however, because a strong decrease in the uranium concentration with time should be evident in the record (at ~30 kyr BP the decrease should be 50%). The data in Table 1 demonstrate that the uranium concentration in the corals is actually rather constant (3-3.5 p.p.m.), in agreement with other published estimates for *A. palmata*[22].

Carbon cycle changes

The ^{14}C-age calculation relies on the assumption that the atmospheric $^{14}C/C$ ratio has not changed with time and therefore can be used to normalize the measured ratio in fossil carbon[35]. Unfortunately, the atmosphere contains less than a ton of ^{14}C and most of the radiocarbon is in the ocean (>40 tons). Subtle changes in the reservoir sizes and/or rates of exchange between reservoirs could have significantly affected the ^{14}C content of the atmosphere. CO_2 measurements in old polar ice showed that the atmospheric concentration was lower during the last glacial than during the Holocene[36-38]. Box-model calculations show that the steady-state $\Delta^{14}C$ would be increased by 25-75‰



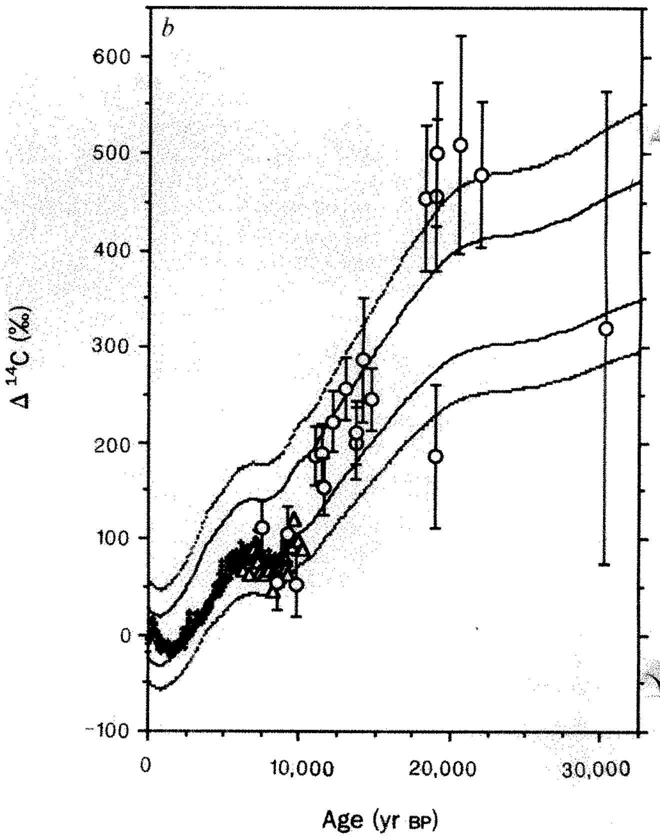


FIG. 3 *a*, Thick line, intensity of Earth's magnetic dipole reconstructed through time (adapted from ref. 48); thin line, cosmogenic nuclide production through time calculated using these palaeomagnetism data and theoretical calculations by Lal[50]. The scale is normalized to unity for present-day values. Using *b*, atmospheric $\Delta^{14}C$ through time reconstructed by the U-Th/^{14}C age relationship (open symbols, the errors are quoted at 2σ). The small crosses correspond to the tree-ring calibration[7] and the open triangles to the Lake of the Cloud calibration (ref. 15, no error has been represented). The two sets of solid lines correspond to the envelopes of $\Delta^{14}C$ expected as a response to changes of the Earth's magnetic field: for those calculations we have assumed constant errors of ±5 and ±10% of the present-day dipole. For sample RGF 9-27-5 we have used the ^{14}C analyses of sample RGF9-27-5 (Geochron) and RGF9-27-4 (Lamont-Doherty). This study suggests that the ^{14}C age of ~17 kyr is anomalous (stratigraphic inversion, see Table 1).

depending on the glacial-to-interglacial CO_2 variation and on the version of the box model used[39-41].

It is possible to increase further the atmospheric $\Delta^{14}C$ by diminishing the rate of penetration of $^{14}CO_2$ in the deep sea. But, to increase the atmospheric $^{14}C/C$ by 400‰ it would be necessary to change dramatically the relative ^{14}C age of the deep waters. By contrast, the mixed layer of the ocean is rapidly exchanging with the atmosphere. A model calculation similar to that of Oeschger *et al.*[42] shows that the difference in ^{14}C between the surface and the deep sea would have to be increased by ~3.5 kyr to account for a 400‰ higher atmospheric $^{14}C/C$ (ref. 27). This age difference is not supported by the palaeoceanographic data on benthic-planktonic pairs of foraminifera dated by ^{14}C AMS. These studies have shown that the ventilation age difference was higher in the Pacific by ~500 ± 100 yr (refs 43, 44) and in the Atlantic by 200 ± 200 yr (ref. 44). Assuming that the highest values measured in the Pacific are valid for the global ocean would lead to a maximal atmospheric $^{14}C/C$ ratio increase of ~70‰.

In summary, changes in the carbon cycle can account for only ~100–150‰ increase in the $\Delta^{14}C$ of the atmosphere as compared to the 400–500‰ inferred from the Barbados data.

Changes in the ^{14}C production

The mean $^{14}C/C$ ratio of the exchangeable carbon on the Earth is maintained by a steady state between production by cosmic rays in the upper atmosphere and radiodecay. The $\Delta^{14}C$ of the atmosphere can therefore undergo variations in response to changes in the flux of galactic protons, solar-wind magnetic properties or the dipole moment of the Earth.

Little is known about possible variations of the cosmic rays on the timescale of interest. As discussed below, the main $\Delta^{14}C$ variations during the Holocene can be explained by changes of the magnetic field and/or changes in the ocean circulation. Therefore it is not necessary to call for modulation of the galactic protons, and this flux can be assumed to be constant with time until data is produced to the contrary.

Changes in the solar-wind modulation are also poorly known but it is important to mention that Raisbeck *et al.*[45] have described brief periods of enhanced cosmogenic production during the past 80 kyr, which they attribute to solar effects. Their work, based on AMS measurements of ^{10}Be in the Vostok ice core, shows that the strongest event occurred at ~35 and 60 kyr BP. In general, the ^{10}Be record obtained in ice cores shows consistently higher concentrations during glacial stades than during warmer periods (a factor of two for the last deglaciation), but the generally accepted interpretation is that precipitation was strongly reduced during the cold periods[46]. Consequently, aside from the solar ^{10}Be spikes, it is extremely difficult to separate the climate signal and the cosmogenic-production signal embedded in the measurements of ^{10}Be in ice cores.

What is well documented, however, is the fact that the magnetic moment of the Earth has varied significantly over the past several thousand years, as first shown by Thellier and Thellier[47]. The palaeointensity record for the past 40 kyr is characterized by two important features: a quasi-sinusoidal variation during the Holocene and a decrease of the moment by a factor of two before 20 kyr BP (refs 48, 49 and Fig. 3*a*).

We converted this dipole history into a ^{14}C production time series by using the calculations of Lal (ref. 50; Fig. 3*a*). The atmospheric ^{14}C production curve cannot be compared directly with the $\Delta^{14}C$ record, however, because the ^{14}C atoms mix continuously in other carbon reservoirs where they ultimately decay with a mean half-life of 8,270 yr. The carbon cycle therefore has a long 'memory', which can easily be simulated by using a simple box model[39,51]. The carbon cycle acts as a low-pass filter: short-term production variations (such as the 11-yr solar cycle) are smoothed out and a phase shift of a few kyr is introduced for the transition between the Pleistocene and the Holocene[39,51]. The results of the calculation are shown in Fig. 3*b*. We assumed no error on the ^{14}C production algorithm[50] and constant errors of 5 and 10% of the present-day magnetic field on the palaeomagnetic record. There is clear agreement between the $\Delta^{14}C$ data calculated from the U–Th/^{14}C comparison and our calculation based on the palaeomagnetic record. The $\Delta^{14}C$ values around 18–22 kyr BP are, however, higher on average than the palaeomagnetism reconstruction, which may indicate that ~100‰ of the signal is the result of reservoir changes associated with the last glacial maximum.

It must be mentioned that some of the palaeomagnetism data compiled by McElhinny and Senanayake[48] were dated by ^{14}C. Using our calibration (Fig. 2) to correct the timescale would only stretch the predicted $\Delta^{14}C$ curve by a few kyr. The U–Th/^{14}C would still be well within the errors of the geomagnetic reconstruction of the $\Delta^{14}C$ curve. Therefore the most reasonable explanation for the large difference between the ^{14}C and U–Th ages involves magnetic modulation of the atmospheric ^{14}C production rate.

Discussion

We have shown here that by using U–Th mass spectrometry one can achieve an age precision and reproducibility comparable to or even better than that obtained by the radiocarbon geochronological method. U–Th ages measured in corals drilled off the shore of Barbados indicate that the ^{14}C timescale is significantly compressed during the late glacial interval. These results can be explained mainly as the consequences of changes in the cosmogenic nuclide production linked to the gradual decrease of Earth's magnetic field[48]. Changes in the carbon cycle[36-39,43,44] can account for ~10–20% of the age discrepancy observed between the two geochronometers. It should be borne in mind that the accuracy of the U–Th ages is proven only back to 10,000 years; nevertheless, all geophysical information available (dendrochronology, varve dating and palaeomagnetism) suggests that our U–Th results are reasonable and that they may be used as a first-order tool to calibrate the radiocarbon timescale beyond the range of the high-resolution dendrochronological comparison. ☐

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1. Fairbanks, R. G. *Nature* **342,** 637–642 (1989).
2. Duplessy, J. C. *et al. Nature* **320,** 350–352 (1986).
3. Bard, E. *et al. Nature* **328,** 791–794 (1987).
4. Broecker, W. S. *et al. Nature* **333,** 156–158 (1988).
5. De Vries, H. L. *Proc. Konink!. Ned. Akad. Wetenshap.* **B61,** 94–102 (1958).
6. Stuiver, M. & Polach, H. A. *Radiocarbon* **19,** 355–363 (1977).
7. Stuiver, M. & Kra, R. *Radiocarbon* **28**, 805–1030 (1986).
8. Lal, D. *Geophys. Monogr. Ser.* **32,** 221–233 (1985).
9. Beer, J. *et al. Nature* **331,** 675–679 (1988).
10. Andree, M. *et al. Clim. Dyn.* **1,** 53–62 (1986).
11. Peng, T. H. *Radiocarbon* (in the press).
12. Suess, H. in *Proc. Conf. Problems Related to Interplanetary Matter* NAS-NSF Publ. 845, Nucl. Sci. Ser. Vol. **33,** 90–95 (NAS-NSF, La Jolla, 1961).
13. Stuiver, M. *J. geophys. Res.* **66,** 273–276 (1961).
14. Stuiver, M. & Quay, P. D. *Science* **207,** 11–19 (1980).
15. Stuiver, M. in *Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 57–70 (Yale Univ. Press, New Haven, 1970).
16. Tauber, H. in *Nobel Symp. 12th, Radiocarbon Variations and Absolute Chronology* (ed. Olsson, I. U.) (Wiley, New York, 1970).
17. Peng, T. H., Goddard, J. G. & Broecker, W. S. *Quat. Res.* **9,** 319–329 (1978).
18. Stuiver, M. *Nature* **273,** 271–274 (1978).
19. Vogel, J. C. *Radiocarbon* **25,** 213–218 (1983).
20. Hammer, C. U., Clausen, H. B. & Tauber, H. *Radiocarbon* **28,** 284–291 (1986).
21. Chen, J. H., Edwards, R. L. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **80**, 241–251 (1986).
22. Edwards, R. L. thesis, California Inst. Technol. (1988).
23. Edwards, R. L., Chen, J. H. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **81,** 175–192 (1987).
24. Edwards, R. L., Taylor, F. W. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **90,** 371–381 (1988).
25. Li, W. X. *et al. Nature* **337,** 534–536 (1989).
26. Dodge, R. E., Fairbanks, R. G., Benninger, L. K. & Maurrasse, F. *Science* **219,** 1423–1425 (1983).
27. Bard, E. *Paleoceanography* **3,** 635–645 (1988).
28. Stuiver, M., Pearson, G. W. & Brazunias, T. *Radiocarbon* **28,** 980–1021 (1986).
29. Cato, I. *Boreas* **14,** 117–122 (1985).
30. Stuiver, M., Kromer, B., Becker, B. & Ferguson, C. W. *Radiocarbon* **28,** 969–979 (1986).
31. Becker, B. & Kromer, B. *Radiocarbon* **28,** 961–967 (1986).
32. Oeschger, H. *et al. Radiocarbon* **22,** 299–310 (1980).
33. Broecker, W. S. & Kaufman, A. *Geol. Soc. Am. Bull.* **76,** 537–566 (1965).
34. Ku, T. L., Ivanovitch, M. & Luo, S. *Quat. Res.* (in the press).
35. Libby, W. F. *Radiocarbon Dating* (Univ. Chicago Press, 1952).
36. Delmas, R. J., Ascencio, J. M. & Legrand, M. *Nature* **284,** 155–157 (1980).
37. Berner, W., Oeschger, H. & Stauffer, B. *Radiocarbon* **22,** 227–235 (1980).
38. Barnola, J. M., Raynaud, D., Korotkevitch, Y. S. & Lorius, C. *Nature* **329,** 408–414 (1987).

39. Siegenthaler, U., Heimann, M. & Oeschger, H. *Radiocarbon* **22**, 177–191 (1980).
40. Keir, R. S. *Earth planet. Sci. Lett.* **64**, 445–456 (1983).
41. Lal, D. & Revelle, R. *Nature* **308**, 344–346 (1984).
42. Oeschger, H., Siegenthaler, U., Schotterer, U. & Gugelmann, A. *Tellus* **27**, 168–192 (1975).
43. Shackleton, N. J. *et al. Nature* **335**, 708–711 (1988).
44. Broecker, W. S. *et al. Paleoceanography* **3**, 659–669 (1988).
45. Raisbeck, G. M. *et al. Nature* **326**, 273–277 (1987).
46. Yiou, F., Raisbeck, G. M., Bourles, D., Lorius C. & Barkov, N. I. *Nature* **316**, 616–617 (1985).
47. Thellier, E. & Thellier, O. *C. r. hebd. Seanc. Acad. Sci. Paris* **212**, 281–283 (1941).
48. McElhinny, M. W. & Senanayaka, W. E. *J. Geomagn. Geoelec., Kyoto* **34**, 39–51 (1982).
49. Tauxe, L. & Valet, J. P. *Phys. Earth planet. Inter.* **56**, 59–68 (1989).
50. Lal, D. in *Proc. 45th Conf. Solar-Terrestrial Relationships and the Earth Environment in the Last Millennia*, 216–233 (Soc. Italiana di Fisica, Bologna, 1988).
51. Houtermans, J. C., Suess, H. E. & Oeschger, H. *J. geophys. Res.* **78**, 1897–1908 (1973).
52. Gillot, P. Y. & Cornette, Y. *Chem. Geol.* **59**, 202–222 (1986).
53. Valladas, H. & Valladas, G. *Coll. Int. CNRS, Habitats du Paléolithique Supérieur* (Roanne–Villerest, in the press).

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The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*

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The dominant mutation *deg-1(u38)* results in a toxic gene product that leads to the late-onset degeneration of a small number of neurons in the nematode *Caenorhabditis elegans*. Both intragenic and extragenic mutations as well as changes in wild-type gene dosage can delay or block the time of onset of the neuronal deaths. The *deg-1* gene has been cloned and a partial complementary DNA reveals that the gene encodes a novel protein that may act as a membrane receptor. Because the late-onset loss of specific sets of neurons, often as a result of dominant mutations, is characteristic of several human neurodegenerative diseases, the analysis of the *deg-1* gene and its suppressors may provide a means of understanding the mechanisms underlying some of these human diseases.

THE selective degeneration of specific classes of nerve cells is characteristic of many inherited human disorders[1] such as Huntington's disease[2,3], familial amyotrophic lateral sclerosis[4], familial Alzheimer's disease[5] and several cerebellar ataxias[6]. Many of these diseases are caused by dominant alleles that result in late-onset degeneration of the affected nerve cells, but their molecular bases are not known. Insights into how genetic lesions can lead to neuronal degeneration could be obtained by studying animal models with similar defects.

We have characterized a dominant mutation *(u38)* of the *C. elegans* gene *deg-1* (*deg*eneration), that results in the degeneration of a small set of neurons. One striking feature of some of these deaths is that they have a late onset, occurring long after the neurons form synapses and become functional. The *deg-1(u38)* mutation results in an abnormal, toxic form of a gene product that is normally non-essential for neuron viability and function. The affected cells swell to many cell diameters and lyse. Because the *deg-1* gene seems to encode a membrane component, cell lysis may result from compromised membrane integrity. The time of the neuronal degeneration depends upon the level of *deg-1(u38)* mutant gene activity, suggesting that the level of accumulation of toxic product, rather than the time of its initial expression, causes the deaths. Mutations in the *mec-6* gene suppress the *deg-1(u38)* neurodegeneration, as well as similar neuronal deaths produced by abnormal toxic forms of the *mec-4* gene product. An understanding of how the mutant *deg-1* and *mec-4* gene products result in neurodegeneration, and of how normal *mec-6* (*mec*hanosensory) activity is required for this degeneration might help in the development of methods for preventing or curing neurodegenerative disorders in humans.

The *deg-1(u38)* phenotype

The *deg-1(u38)* mutant was identified in a screen for touch-insensitive mutants following ethyl methanesulphonate (EMS) mutagenesis at 25 °C (ref. 7). The gene is located on the X chromosome (see legend to Fig. 1). The *deg-1(u38)* animals differ from other touch-insensitive mutants[7,8] in that although both types of mutants are insensitive to the gentle touch of a hair, only the *deg-1* mutants are insensitive to the more severe prod of a thin wire. They are also insensitive only at the tail. This more severe phenotype (designated Tab for Touch abnormal) suggests a defect in the two PVC interneurons, which receive synaptic inputs from posterior touch receptor neurons[9]. The PVC cells degenerate in these mutants. A similar behavioural abnormality results from laser ablation of the PVC cells in wild-type animals (W. W. Walthall and M.C., unpublished data).

Although the PVC cells arise during embryogenesis[10], *deg-1* mutants are touch-sensitive at hatching and become Tab later, during the second and third larval stages (L2 and L3) at 25 °C (Fig. 1). The stage of onset of touch insensitivity is delayed further when animals are grown at lower temperatures, so mutants grown at 15 °C become Tab as gravid adults.

Virtually all *deg-1* animals, when viewed by Nomarski microscopy[11], have one or two degenerating cells near the normal position of the PVC cells, 24 h after hatching at 25 °C (Fig. 2). The degeneration is first seen as a small vacuole that surrounds the nucleus. This vacuole, which contains particles displaying Brownian motion, enlarges by several cell diameters over the next few hours, during which the nucleus disintegrates.

This degeneration differs from programmed cell death, a common feature in *C. elegans* development, in which affected cells become refractile and condensed as they die[11]. Here we use the term degeneration for the type of death seen in *deg-1(u38)* mutants to distinguish it from the morphologically distinct programmed cell death. The *deg-1(u38)* degeneration phenotype (termed Deg) has been seen in animals with dominant mutations of the gene *mec-4* (*mec-4(d)*). Dominant, but not recessive, *mec-4* mutations result in the degeneration of the touch receptor neurons[7,8], probably by the production of a toxic product that is expressed within the touch receptor neurons[12]. As in *deg-1(u38)* animals, some of the affected cells in *mec-4(d)* mutants display a late-onset degeneration[8,13].

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Other classes of neurons, unrelated by position, lineage, or function to each other or to the PVC cells, also degenerate at various times during development in *deg-1(u38)* mutants (Fig. 2). Although all of the animals become Tab and most have one or two degenerating PVC cells at 24 h at 25 °C, the pattern of these other deaths varies considerably (Fig. 2 legend). This variability may reflect incomplete penetrance of the Deg phenotype for each cell or variations in the times of onset or duration of the degenerations.

The *deg-1* mutants also contain empty vacuoles (vacuoles not associated with nuclei) at the tip of the nose, along the ventral cord, and in the tail (Fig. 2), especially in newly hatched animals. They may be located in the hypodermis (epidermis), but whether they result from *deg-1* expression in this tissue or are a response to neuronal damage is unknown.

Genetic characterization

Alleles like *u38* seem to be extremely rare, as no similar *deg-1* mutation has yet been isolated. The original *u38* mutant was isolated in a screen of progeny representing 70,000 copies of the gene[7]. Such rarity (loss-of-function mutations in *C. elegans* arise at a frequency of $\sim 5 \times 10^{-4}$ (refs 14, 15)) and the dominant Deg and Tab phenotypes suggest that *u38* is not a *deg-1* null mutation. Indeed, loss of *deg-1* function gives rise to no detectable abnormal phenotype (the animals appear wild type) and the *u38* mutation seems to encode an abnormal product that interferes with normal cell activity.

The *u38* animals can readily be reverted to a wild-type phenotype. As 24 of 25 non-Tab (touch-sensitive) revertants obtained by EMS mutagenesis have not segregated Tab animals, these reverting mutations are presumed to be intragenic. (The remaining revertant strain produces some Tab progeny but with an unusual segregation pattern and has not been studied further.) Three additional intragenic revertants arose spontaneously from a *mut-2; deg-1* double mutant (the *mut-2* mutation often activates transposons in *C. elegans*[16]). Most of the suppressor mutations, including those obtained in the *mut-2* background, seem to revert almost completely the Tab and Deg phenotypes, and all but one are recessive to the *u38* mutation. The frequency at which the intragenic suppressor mutations arose with EMS mutagenesis (4×10^{-4}) is similar to that found for loss-of-function mutations in other *C. elegans* genes[14,15]. The molecular analysis below also supports the hypothesis that disruption of the *deg-1* gene leads to a wild-type null phenotype.

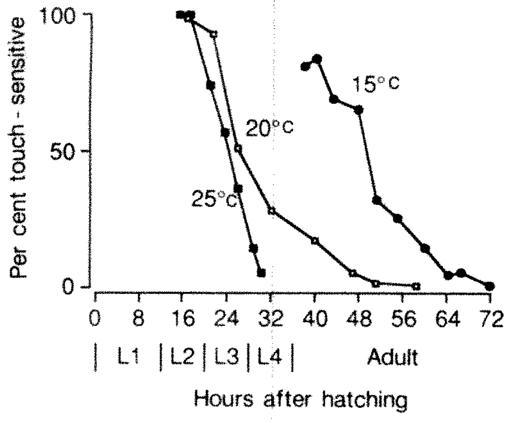

FIG. 1 Onset of the *deg-1* touch-insensitivity (Tab) phenotype at various temperatures. The abscissa records time in 25 °C-hour equivalents (*C. elegans* grows at approximately twice the 15 °C rate at 25 °C).
METHODS. Strains were maintained on *Escherichia coli* strain OP50-1 as described[14,56]. Animals from strains grown at the indicated temperatures for at least two generations were synchronized at hatching by washing hatched animals and bacteria from the plates with M9 buffer[14] and collecting the larvae that hatched from the remaining eggs in the next 1–2 h. At the indicated times animals were tested for touch sensitivity at the tail by touching with a thin hair[8]. At least 100 animals were examined at each temperature. The *deg-1(u38)* mutation maps within 0.014 map units of the *mec-7* on the X chromosome by the following criteria: no wild-type progeny were found among 3,533 total progeny from *+[+deg-1(u38)]/lon-2(e678) [mec-7(e1506)+]* heterozygotes nor among 5,334 and 5,960 heterozygote progeny from, respectively, *+[+deg-1(u38)] dpy-6(e14)/dpy-7(e88) [mec-7(e1506)+]+* and *dpy-7 [+deg-1(u38)]+/+[mec-7(e1506)+] dpy-6(e14)* heterozygotes. These other mutations are described in refs 11 and 14. *deg-1* loss-of-function mutations complement the recessive *mec-7* allele *e1506* for touch insensitivity, an indication that the two loci are different genes.

The high frequency of intragenic *deg-1* revertants that do not produce a detectable phenotype suggests that the wild-type *deg-1* product is not essential. The gene could be nonessential because of a marginal effect of *deg-1(+)* in the wild type or the replacement of its function by the products of other genes, either of the same gene family[15,17] or of a redundant genetic pathway (for example, ref. 18). Whereas the loss of a constituent of a gene family or one pathway might not result in observable defects, a mutation leading to the production of an abnormal, interfering gene product could. The molecular analysis below suggests that a *deg-1* gene family exists, but the possibility of a redundant pathway cannot be excluded.

Two of the intragenic suppressor mutations, *u352* and *u354*, only partially suppress the effects of *u38*. They delay the onset of the Tab phenotype, by about 24 and 48 h at 25 °C for *u354* and *u352*, respectively (Fig. 3). The PVC deaths are also seen later, the cells dying in young and old adults, respectively. The various deaths caused by the *u38* mutation may be unequally affected by these suppressor mutations; in particular the *u352* mutation seems to have its most pronounced effect on the PVC death (Fig. 3).

Gene dosage experiments suggest that the *u38* mutation results in an abnormal gene product that interferes with cell function, rather than in the overproduction of a normal cell product. Additional copies of the wild-type gene delay, although to a smaller degree than temperature or intragenic mutations, the onset of the *u38* Tab phenotype (Fig. 4). In general, the delay in the onset of the Tab phenotype increases in proportion to the ratio of wild-type to mutant allele.

Independence of *deg-1(u38)* degenerations

The *deg-1* degenerations differ from programmed cells deaths not only morphologically, but also genetically. Mutations that prevent all programmed cell deaths in *C. elegans* hermaphrodites (*ced-3(n717)* and *ced-4(n1162)*; ref. 19) did not prevent the *deg-1* degenerations. In fact, *ced-3*; *deg-1(u38)* and *ced-4*; *deg-1(u38)* double mutants had additional degenerations in the head at hatching (total deaths with *ced-3*: 4.0 ± 0.4; with *ced-4*: 4.6 ± 0.6; mean ± s.e.m., $n = 25$ for each; *deg-1(u38)* mutants alone have 2.2 ± 0.2 deaths, $n = 20$). These data suggest that some of the extra cells that normally survive in *ced-3* and *ced-4* animals live and then degenerate in the corresponding *deg-1* double mutant. This supports the hypothesis[19,20] that cells prevented from undergoing programmed cell death can differentiate, in this case at least enough to express the Deg phenotype.

One explanation for the late-onset degeneration of the PVC cells is that larval cell interactions trigger the deaths, perhaps by initiating aberrant differentiation or by toxically stimulating the cells, as in glutamate neurotoxicity[21]. Many neurons arise post-embryonically in *C. elegans*, but they do not seem to be required for the PVC deaths, as the double mutant *lin-6(e1466)*; *deg*-1(*u*38), in which none of these cells are made[22,23], is Tab. Moreover, the elimination of the touch receptor neurons, which synapse onto the PVC cells, by the addition of the *unc-86(e1416)* mutation[8] had no effect in the *deg-1* deaths. Thus, neither postembryonically derived targets nor a major input to the PVC cells is required for these deaths. Together with the different timing and position of the degenerations, these data suggest that the *deg-1(u38)* mutation may act in a cell-autonomous fashion, although they do not rule out the possibility that cell interactions could trigger a *deg-1*-dependent degeneration. Herman[12] has suggested the *mec-4(d)* mutation also acts cell-autonomously.

Suppression by *mec-6* mutations

Mutations in the *mec-6* gene suppress the degenerations caused by both the *mec-4(d)* and *deg-1(u38)* mutations. This result not only indicates an underlying genetic similarity in the deaths caused by these mutations, but also identifies a gene, *mec-6*, whose activity is required for the neurodegenerative process. The effect on the *mec-4(d)* mutations was discovered through the construction of a set of double mutants, each containing a *mec-4(d)* mutation and a mutation in one of the other genes required for touch receptor function[7] to determine whether any of these mutations could prevent the *mec-4(d)* deaths. (Previous experiments (ref. 8, and unpublished results) showed that mutations in genes required for the generation of the touch receptors or for the specification of their differentiation prevent the appearance of the *mec-4(d)*-dependent deaths.) Mutations in the genes *mec-1*, *mec-2*, *mec-6* to *mec-10*, *mec-12*, *mec-14*, *mec-17* and *mec-18* were tested, but only *mec-6* mutations suppressed the *mec-4(d)* degenerations. Although the animals remained touch insensitive (the *mec-6* phenotype), the touch cells did not die. In 14 *mec-6–mec-4(d)* strains made using eight *mec-6* alleles and the three *mec-4(d)* mutations, all had normal appearing adult touch cells. The *mec-6* mutations suppressed only when homozygous. The *mec-6* mutation *u247* results in a temperature-sensitive phenotype for touch sensitivity[7]. Suppression of the *mec-4(d)* degenerations by *u247* is also temperature sensitive: deaths were seen in newly-hatched larvae at 15 °C, but not at 25 °C (it was not possible to delay the onset of these deaths in temperature-shift experiments). These experiments suggest that the loss of *mec-6* activity, not the production of an unusual, allele-specific product, results in suppression. As other features of the touch receptors seem unaffected in *mec-6* mutants[7,8], the wild-type *mec-6* product is probably required for neurodegeneration induced by the abnormal products of the *med-4(d)* alleles.

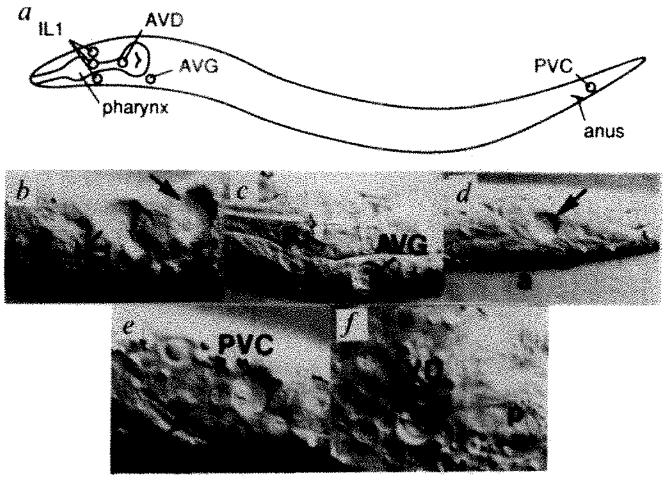

FIG. 2 Defects in *deg-1* mutants. *a*, Schematic diagram of the left side of newly hatched *C. elegans* larva indicating the positions of some of the cells affected by the *deg-1(u38)* mutation. All of these neurons, except AVG, have homologues on the other side of the animal. These cells die at different times: the IL1 sensory neurons and the AVG interneuron die at hatching, the PVC cells in the third larval stage (L3), and the AVD interneurons near the end of the last (L4) larval stage. Other degenerations are sometimes seen. Because the degenerations can be quite large, distorting the surrounding pattern of nuclei, it is difficult to identify unambiguously the affected cells. The degeneration pattern in *u38* males does not differ substantially from that of hermaphrodites. *b*, Differential interference contrast micrographs of *deg-1* degenerative deaths (IL1 and large arrow) and programmed cell deaths (small arrows) posterior to the first bulb of the pharynx (p) in a newly-hatched *ced-1*; *deg-1* double mutant. (The *ced-1* mutation *e1735* delays the engulfment of cells dying by programmed cell death[57].) The two types of deaths are morphologically distinct. The cells here and in subsequent photomicrographs have been tentatively identified by criteria given below. Not all of the IL1 cells die: one to three IL1 cells (wild type have six) were seen in six *deg-1* adults examined by serial section electron microscopy[58,59]. Magnification, ×800. *c*, Putative AVG death in a newly-hatched larva. 24% (12/49) newly hatched animals had this death. *d*, Empty vacuole in the tail of a newly hatched larva. a, Anus. *e*, PVC death in 24 h larva. *f*, Putative AVD death at the time of the last larval moult (36 h). Criteria for identifying cells: The dying cells were identified by their positions[59] and the following data. IL1: Cells with the characteristic rootlet[59] of the IL1 cells were missing in serial sections of the tip of the nose of adult animals. AVG: A single degeneration is seen in the retrovesicular ganglion just posterior to the rear bulb of the pharynx in many newly hatched animals. AVG is the only unpaired neuron in this region[60]. AVD: This pair of interneurons helps mediate anterior touch sensitivity[9], but since the anterior touch circuit also utilizes other interneurons, loss of the AVD cells in the *deg-1* mutants does not produce a Tab phenotype. However, laser ablation of the AVA interneurons in *deg-1* animals results in animals that are virtually incapable of backward movement as young adults but not as larvae (J. Thomas, personal communication), a result seen in wild-type animals when both the AVA and AVD cells are ablated[9]. PVC, See text.

Mutations in *mec-6* (*u41*, *u247* and *u450* were used) also suppress the effects of *deg-1(u38)*. Double mutants are insensitive to the touch of hair at both head and tail (the *mec-6* phenotype), but they are not Tab. Moreover, the *mec-6* mutations seem to suppress all of the *deg-1*-induced deaths (no vacuolated cells were seen in any newly-hatched larvae) as well as the appearance of the presumptive hypodermal vacuoles (Fig. 2*d*). As with the suppression of the *mec-4(d)* deaths, suppression only occurred in *mec-6* homozygotes. Suppression of *deg-1* by *mec-6* indicates that *mec-6* expression is not restricted to the touch system, even though the only detectable behavioural phenotype of *mec-6* mutants is touch insensitivity. The absence of a Tab phenotype in *mec-6* mutants suggests that *deg-1* in the PVC cells is not replaced by the activity of other *deg-1*-like genes, at least not those requiring *mec-6* function.

Molecular analysis of *deg-1*

The *deg-1* gene was cloned by transposon tagging, utilizing the *mut-2*-derived revertants (Fig. 5). DNA flanking the insertion site of the transposon Tc1 hybridized to C47C12 (Fig. 6), a cosmid clone adjacent to a cosmid containing the *mec-7* gene[24,25], a position consistent with the genetic mapping of *deg-1*

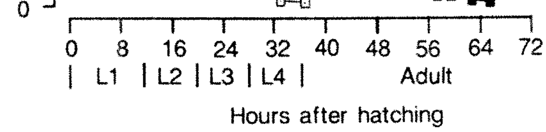

FIG. 3 Onset of the Tab phenotype in *deg-1(u38)* and two partially reverted strains [*deg-1(u38u354)* and *deg-1(u38u352)*]. Animals were grown from synchronized populations at 25 °C (see Fig. 1). At least 100 animals of each strain were examined. The Tab phenotype, as in *u38* mutants, is temperature dependent in *u38u352* and *u38u354* animals (data not shown). Not all *deg-1*-dependent deaths seem to be affected in the same way by these suppressor mutations. The number of degenerations in newly hatched animals is reduced in *u38u354* animals (0.6 ± 0.2 deaths) but not in *u38u352* animals (2.9 ± 0.3 deaths; $n = 20$ for each; *u38* animals have 2.2 ± 0.2 deaths). Both suppressor-containing strains produce animals with the presumptive AVD deaths (Fig. 2) at 36 h (5/11, 3/11 and 2/10 for *u38*, *u38u352* and *u38u354*, respectively).

(Fig. 1 legend). Hybridization with C47C12 revealed insertions in one other *mut-2*-derived revertant and a possible deletion in one EMS-derived revertant (Fig. 5). Further evidence that the transposon insertions are in the *deg-1* gene came from experiments in which the transposon-containing strains were themselves reverted by reintroducing the *mut-2* mutation. This reversion with the reappearance of Deg and Tab phenotypes was accompanied by the excision of the transposon (Fig. 5).

Two partial *deg-1* cDNAs were isolated from a λgt10 library with cDNAs from 12-hour-old larvae, provided by J. Ahringer and J. Kimble (University of Wisconsin). As the transposon is inserted in the revertant *u38 u478* residues in a 187-bp region that contains sequences in the cDNAs surrounding a 50-bp intron (see Fig. 6 legend), it is likely that these cDNAs represent *deg-1* transcripts. The larger cDNA encodes an open reading frame of 884 base pairs (bp; Fig. 7) followed by 141 bp and a poly(A) tail. The smaller cDNA lacks 72-bp from within the open reading frame. As no untranslated sequence precedes these open reading frames, the cDNAs seem to be incomplete. The inferred polypeptide sequence of 294 amino acids from the larger cDNA, which is not strongly homologous to other DNA and protein database entries, has three notable features: (1) a hydrophobic region from residues 215–255; (2) two possible sites for *N*-linked glycosylation[26] located at positions 8 and 122; and (3) a cysteine-rich region, containing 13 of the 14 cysteines in the sequence, between positions 66 and 187. The 72 bp absent in the smaller cDNA encode 24 amino acids in the cysteine-rich region (including two cysteines) and could represent an alternatively-spliced variant.

The structure of the *deg-1* product is consistent with its being a membrane protein. The hydrophobic domain has an uninterrupted stretch of 17 amino acids, which is sufficient to span the lipid bilayer[27], and an additional 24 amino acids, only three of which are charged. This structure but not sequence, is also found in the predicted membrane spanning domain of the β subunit of the T-cell receptor[28,29]. It is possible that the extended hydrophobic region of the *deg-1* product (including the charged residues) is submerged in the cell membrane, and may interact with analogous domains of other membrane proteins. The localization of the large number of cysteines, a feature of several receptor proteins[30–33], and the two *N*-linked glycosylation sites in the *N*-terminal region, is consistent with this region being an extracellular domain (Fig. 7).

The molecular analysis supports the hypothesis derived from our genetic studies that *deg-1* may be a member of a gene family. The cDNAs as well as a genomic DNA fragment to which they bind (the IC fragment; Fig. 6) hybridize to several genomic fragments (Fig. 5*b*).

The wild-type *deg-1* gene, as defined by the restriction fragments that differ in the *u38* revertants or that hybridize with the cDNAs, is contained within a 35 kilobase (kb) *Bam*HI fragment (Fig. 6). The corresponding fragment cloned from the dominant allele *deg-1(u38)* (Fig. 6, TU#3), when transformed into wild-type animals, produces both the Tab and Deg phenotypes (Fig. 6 legend). Most of the transformant lines are unstable and seem to have extrachromosomal arrays of the injected DNA. However, in one strain (*uIn1*) the mutant phenotypes are stably inherited, and karyotype and Southern blot analysis indicate that at least ten intact copies of the transformed DNA have attached to the X chromosome.

The *uIn1* animals resemble *u38* mutants, but display some differences. The PVC neurons degenerate at essentially the same time in both strains (data not shown), but more degenerating cells are seen in the head during the second larval stage (L2) in the transformed animals (0.5 ± 0.0 deaths in *u38* ($n = 21$) and 3.7 ± 0.2 in *uIn1* ($n = 9$)). The extra copies of *deg-1(u38)* DNA or its misexpression in the transformants may cause additional cells to die or change the onset of some cell degeneration. Unlike the situation in *u38* mutants, the Deg and Tab phenotypes of *uIn1* animals are not completely suppressed by *mec-6* mutations *u450* and *e1342*. The head degenerations in *mec-6*; *uIn1* strains are suppressed, but the PVC degenerations occur in many of the animals (8 of 28 late L2 *mec-6*; *uIn1* larvae had these deaths compared with 7 of 20 *uIn1* larvae of the same age). Most *mec-6*; *uIn1* adults are Tab. Hence, some *deg-1*-dependent degenerations do not absolutely require wild-type *mec-6* function, at least under these conditions of increased copies of the *u38* DNA.

Discussion

Dominant mutations of *deg-1* and *mec-4* cause the degeneration of a small number of neurons. Although different cells die in the *deg-1* and *mec-4* mutants, these mutations cause an apparently similar cell death that differs morphologically and genetically from programmed cell death. The degeneration result from the expression of alleles that seem to encode abnormal products; the wild-type genes are not needed for cell viability. The dominant mutations are suppressed by *mec-6* mutations, suggesting that similar molecular processes may underlie these neuronal degenerations. Over 420 mutations (many of them dominant) in 18 genes needed for touch receptor development and function have been identified, yet only the three *mec-4(d)* mutations cause the deaths of the touch cells[7], and no other mutations are known that mimic the *deg-1(u38)* mutation. Perhaps only a few cellular processes can be disrupted to cause this type of selective neurodegeneration. (This is not the only type of abnormal cell death seen in *C. elegans*; mutations in *egl-1*[34], *lin-24*[35] and *lin-33*[35] seem to produce ectopic cell deaths.)

An intriguing feature of the *deg-1(u38)* phenotype is the late onset of cell degeneration of many of the affected cells. Because the loss of the PVC cells results in a detectable phenotype, the behavioural defect in these animals also seems to be of late onset, even though other cells have died earlier. The PVC degenerations can occur in animals of different ages (from second stage larvae to egg-laying adults) depending on the nature of the mutation, the growth temperature, and the dosage of the wild-type gene. These data suggest that the degenerative

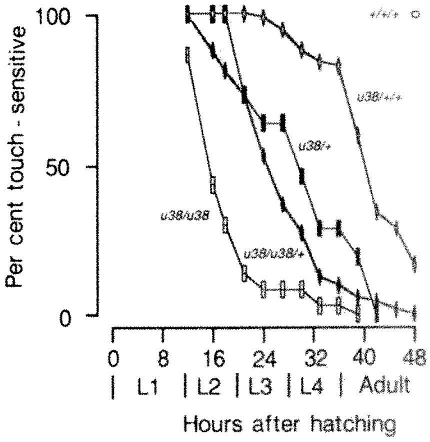

FIG. 4 Onset of the Tab phenotype in animals with different doses of the wild-type (+) and mutant (*u38*) alleles of *deg-1*. Progeny of hermaphrodites with the genotype *stDp2/+; deg-1(u38)+dpy-6(e14)/unc-18(u81)+dpy-6(e14)* were synchronized at hatching (Fig. 1 legend). At the indicated times after hatching animals that were Tab were plated individually so that their progeny could be examined so as to determine their genotypes. All animals that have three copies of the region (*u38/u38/+* (filled diamonds), *u38/+/+* (open diamonds) and *+/+/+* (open circle)) contain *stDp2*, a duplication of the *dpy-6 deg-1* region of the X chromosome that has been translocated to chromosome II and is lethal when homozygous (R. Waterston, pers. comm.). All these animals are wild type in length. At least 50 animals of each of these genotypes were examined. Animals with two copies of the region (*u38/u38* (open bars) and *u38/+* (filled bars)) are homozygous for the *dpy-6* mutation and are shorter in length (Dpy). Thirty-seven *u38/u38* and eleven *u38/+* animals were examined. The Dpy phenotype does not seem to affect the onset of the degeneration (compare Fig. 1). (The *dpy* and *unc* mutations are described in ref 16.) Wild-type animals (+/+; not shown), like the +/+/+ animals, are touch-sensitive at all times.

phenotype is not directly coupled to a specific, developmental event, such as the appearance or maturation of a particular set of cells, a conclusion supported by the observations that the *deg-1*-dependent PVC deaths are not affected when various synaptic targets and inputs have been eliminated genetically. More likely, the accumulation of a mutant product leads to the degeneration phenotype. Certainly, a particular time of onset cannot be taken as a characteristic feature of the Tab phenotype. Such may also be true of human diseases. For example, the study of restriction fragment length polymorphisms linked to the Huntington's disease gene suggested that mutant alleles of the same genetic locus may give rise to diseases with different times of onset[36].

One possible explanation for the marked effect of *deg-1* and *mec-4* mutations on specific nerve cells, as well as the vacuolization phenotype, is that the genes encode membrane components such as ion channels or receptors, that are required by subsets of neurons. An abnormal product from a dominant mutation might prove toxic if, directly or indirectly, it compromised membrane integrity. Several observations suggest that chronically open membrane channels can lead to cell lethality. The continued opening of acetylcholine channels at the neuromuscular junction, for example, leads to localized degeneration of the endplate because of the activation of calcium-dependent proteases[37], and sustained opening of capsaicin-sensitive channels in putative pain receptor neurons can cause cell lysis through osmotic imbalance or calcium influx[38]. The osmotic disruption and calcium entry that result from glutamate neurotoxicity[39] may also result from a similar mechanism. The partial sequence of the *deg-1* product is consistent with a role in membrane function, although it is not homologous to known channel proteins. The *deg-1* product could affect membrane integrity indirectly. For example, the mutant product might bind to a channel or other membrane component and modify its activity. Of relevance may be a mammalian protein with only one apparent transmembrane domain that confers novel channel properties on *Xenopus* oocytes[40,41]. It is not known whether this

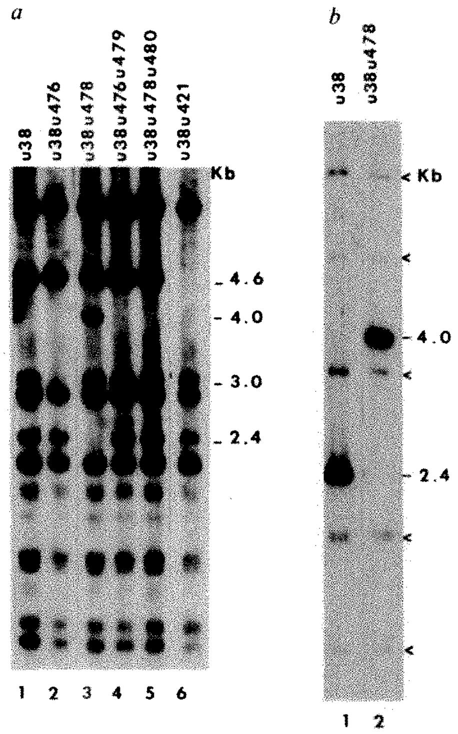

FIG. 5 Southern blot analysis of *deg-1* DNAs. *a*, Genomic DNA digested with *Eco*RI and *Xba*I and probed with cosmid C47C12. Lane 1, *deg-1(u38)*; lane 2, a *mut-2*-derived revertant of *deg-1(u38)* containing a Tc1 insertion in the 3.0 kb *Eco*RI–*Xba*I fragment (fragment I in Fig. 6; the resulting fragment with the insertion comigrates with a second fragment and increases the intensity of the band at 4.6 kb); lane 3, a second revertant with an insert in the 2.4 *Eco*RI fragment (fragment IC in Fig. 6); lanes 4 and 5, excision strains from *deg-1(u38u476)* and *deg-1(u38u478)*, respectively, which express the Deg and Tab phenotypes; lane 6, an EMS-derived revertant missing the 4.5 kb *Eco*RI fragment (fragment D in Fig. 6). No differences in the hybridization pattern were detected between *deg-1(u38)* and wild-type DNA probed with cosmid C47C12 or R02A8 (see Fig. 6). A third *mut-2*-derived revertant, *u38u477*, also had the same pattern of hybridization as the wild type. *b*, Genomic digested with *Eco*RI and *Xba*I and probed with fragment IC. The IC fragment hybridizes to the *deg-1* cDNA and contains the insertion site of the Tc1 transposon in revertant strain *u38u478*. Lane 1, *deg-1(u38)*; lane 2, *deg-1(u38u478)*. Five cross-hybridizing bands, which are also seen in the wild type in other blots, are indicated by arrowheads.

METHODS. *C. elegans* genomic DNA was prepared as described[61]. General molecular methods were as described[62], with final washes of blots in 0.1 × SSC at 65 °C. Initially *deg-1* DNA was cloned from the *u38u476* strain. Southern blots of DNA from this strain, when probed with DNA from the transposon Tc1[61], revealed a single, novel Tc1-containing *Eco*RI–*Xba*I fragment that cosegregated with the non-Tab phenotype in recombinants with *u38*. This fragment was isolated from a plasmid library constructed of size-fractionated *Eco*RI–*Xba*I fragments from *u38u476* DNA in plasmid pUC18. The resulting plasmid was digested with *Eco*RV to remove the Tc1 element and religated to produce plasmid TU#8.

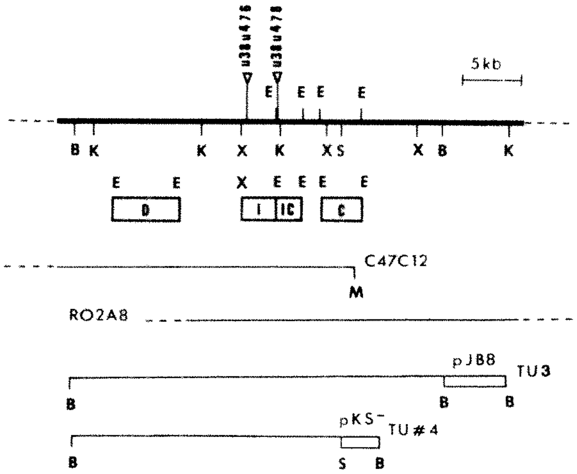

FIG. 6 DNAs of the *deg-1* region. The top line represents a partial restriction map of genomic DNA. Triangles denote the approximate positions of transposon insertions in the indicated revertants (all insertions are 1.6 kb, the size of the transposon Tc1, but the presence of this transposon has been confirmed only in strain *u38u476*). The boxes under the restriction map indicate fragments possessing DNA rearrangements in *u38* revertants or that hybridize to *deg-1* cDNAs: D is deleted in *u38u421*; I contains the Tc1 insertion in *u38u476*; IC contains the transposon insertion in *u38u478* and hybridizes to the *deg-1* cDNAs (the transposon resides in a 187 bp *Eco*RI–*Nde*I fragment at the 5′ end of IC; the sequence of this fragment is identical with the *deg-1* cDNAs, except for a 50 bp intron 160 bp from the *Eco*RI site); and C is a fragment that also hybridizes to the cDNAs. C47C12 and R02A8 are cosmids containing wild-type DNA[24]. TU#3 is a cosmid clone of the *Bam*HI fragment from *deg-1(u38)*; when transformed into wild-type animals, TU#3 produces the Deg and Tab phenotypes. TU#4 is a derivative of TU#3 that lacks the 3′ *Sma*I–*Bam*HI fragment and does not generate the Deg or Tab phenotypes on transformation into wild type. B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; S, *Sma*I; X, *Xba*I; M, *Mbo*I; not all *Eco*RI, *Xba*I and *Mbo*I sites are indicated.

METHODS. Genomic subclones of wild-type DNA from C47C12 were made by ligation into pUC18. The TU#3 cosmid was obtained by isolating large fragments from *Bam*HI-digested *deg-1(u38)* DNA from a sucrose gradient[62] and ligating them to the *Bam*HI–*Hin*dIII and *Sal*I–*Bam*HI fragments of the cosmid vector pJB8[63]. This library was screened with all four genomic fragments (D, I, IC and C) individually as probes. Animals were transformed with TU#3 DNA by co-injection of the DNA into wild-type oocytes[64] with either pPD10.41 or pGB3.5, plasmids that contain an antisense construct of the *C. elegans unc-22* gene that produces a twitching phenotype (A. Fire, personal communication). About half of the twitcher transformants also segregated animals that were Tab and Deg. *uln1* resulted from co-injection of TU#3 and pGB3.5 and showed linkage with *unc-18(e81)* on the X chromosome. Transformations with TU#4 were done similarly; Southern blotting confirmed that TU#4 was present in the transformed animals.

```
1          10            20            30            40           50           60
GA ATT CGT GTC CTT CTA TTC GTA AAC ACA TCA GAT TAT ATG TCA ACT TCT GAG TCA TCC GGA
   Ile Arg Val Leu Leu Phe Val Asn Thr Ser Asp Tyr Met Ser Thr Ser Glu Ser Ser Gly
   1                                   10                                      20

           70            80            90            100          110          120
   GTT CGA CTG GCC ATC CAT CCA CCA ACT GAG TAC CCG TTC CCC GAC ACA TTC GGC TAT TCT
   Val Arg Leu Ala Ile His Pro Pro Thr Glu Tyr Pro Phe Pro Asp Thr Phe Gly Tyr Ser
   21                                  30                                      40

           130           140           150           160          170          180
   GCG CCA GTT GGT TTT GCA AGT AGT TTT GGA ATC AAA AAG AAA GTG ATG CAA AGG TTG CCA
   Ala Pro Val Gly Phe Ala Ser Ser Phe Gly Ile Lys Lys Lys Val Met Gln Arg Leu Pro
   41                                  50                                      60

           190           200           210           220          230          240
   GCA CCA TAT GGA GAA TGT GTA GAA ACG AAG AAA GTT GTA GAC AGA AAT TAT ATT TAC GCG
   Ala Pro Tyr Gly Glu Cys Val Glu Thr Lys Lys Val Val Asp Arg Asn Tyr Ile Tyr Ala
   61                   *              70                                      80

           250           260           270           280          290          300
   GGG TAC GAT TAT CAT CCA GAA GGT TGT CAT AGA AGT TGC TTC CAA AAT GGA CTG ATT GAT
   Gly Tyr Asp Tyr His Pro Glu Gly Cys His Arg Ser Cys Phe Gln Asn Gly Leu Ile Asp
   81                               *  90           *                         100

           310           320           330           340          350          360
   GAT TGT TCG TGT GGA GAT CCT CGT TTC CCA GTA CCA GAA GGT TAT AGA CAT TGC TCG GCA
   Asp Cys Ser Cys Gly Asp Pro Arg Phe Pro Val Pro Glu Gly Tyr Arg His Cys Ser Ala
   101  *       *                      110                              *     120

           370           380           390           400          410          420
   TTT AAT GCA ACA GCT CGT ACC TGT CTT GAG AAG AAC ATT GGC TCA GTT GGA GAT TTC CAT
   Phe Asn Ala Thr Ala Arg Thr Cys Leu Glu Lys Asn Ile Gly Ser Val Gly Asp Phe His
   121                          *      130                                    140

           430           440           450           460          470          480
   CAT ATC ACT CAA AAA ATG GAC AAA TGC GTG TGT AAG CAA TCA TGT GAA GAA ATT ATT CAT
   His Ile Thr Gln Lys Met Asp Lys Cys Val Cys Lys Gln Ser Cys Glu Glu Ile Ile His
   141                              *  150  *               *                 151

           490           500           510           520          530          540
   GAA GTT ACC TTT TCA TGC TCC AAA TGG CCT TCG GGA GCT ACT GAC[CTT GGA GAC TGT GAT
   Glu Val Thr Phe Ser Cys Ser Lys Trp Pro Ser Gly Ala Thr Asp[Leu Gly Asp Cys Asp
   161                  *              170                                  * 180

           550           560           570           580          590          600
   GGT ATG ACA GAA AGC GAG TGC GAA CAA TAC TAT CGG CTA AAT GCG GCA ATG ATC GAG]GTA
   Gly Met Thr Glu Ser Glu Cys Glu Gln Tyr Tyr Arg Leu Asn Ala Ala Met Ile Glu]Val
   181                      *          190                                    200

           610           620           630           640          650          660
   TTC TAC GAA CAA CTG AAC TAC GAA CTG CTT CAA GAA TCA GAG GCA TAC GGT TTG GTT AAC
   Phe Tyr Glu Gln Leu Asn Tyr Glu Leu Leu Gln Glu Ser Glu Ala Tyr Gly Leu Val Asp
   201                                 210                                    220

           670           680           690           700          710          720
   TTG ATC GCC GAT TTT GGA GGA CAT TTA GGA CTT TGG CTA GGA TTC TCC GTA ATC ACC GTG
   Leu Ile Ala Asp Phe Gly Gly His Leu Gly Leu Trp Leu Gly Phe Ser Val Ile Thr Val
   221                                 230                                    240

           730           740           750           760          770          780
   ATG GAA GTT TGT GTT CTG CTT GTT GAT ATG ATT TCC CTT TTC TTT AAA AGT CGG CAC GAA
   Met Glu Val Cys Val Leu Leu Val Asp Met Ile Ser Leu Phe Phe Lys Ser Arg His Glu
   241          *                      250                                    260

           790           800           810           820          830          840
   GAA AAA CTT CTG AGA CAG AGC ACA AAA AGG AAA GAT GTT CCA GAA GAT AAA CGG CAA ATT
   Glu Lys Leu Leu Arg Gln Ser Thr Lys Arg Lys Asp Val Pro Glu Asp Lys Arg Gln Ile
   261                                 270                                    280

           850           860           870           880          890          900
   ACA GTT GGA TCA GGG CGA AAG TCA GAC GCT TTC GTA TCA ATA TAA ATA CCA ACT CTC TTT
   Thr Val Gly Ser Gly Arg Lys Ser Asp Ala Phe Val Ser Ile STOP
   281                                 290

           910           920           930           940          950          960
   TGA ACA ACT ATT ATA TAA CGT TAA TTT TGA ACT GGG TTT CTC AAG ATG TAG TAT ACA ATG

           970           980           990           1000         1010         1020
   CTG TAA CAC GTT TCA CCT TCA TTC GTT TTT TCC GAT CTC TAA TTG TAT ATA GTG AGC TTT

   TTG ATT A90
```

FIG. 7 DNA and predicted protein sequence of *deg-1* cDNA. The sequence of the larger cDNA is shown. The smaller cDNA lacks the DNA encoding residues 176–199 (bracketed). Potential glycosylation sites[26] are underlined, cysteine residues are asterisked, and the hydrophobic region is double-underlined.

METHODS. The cDNAs were subcloned from λgt10 into the *Eco*RI site of pKS$^-$ and pKS$^+$ (Stratagene). Single stranded DNA was rescued from a set of nested deletions[65] using helper phage R408 (Stratagene) and sequenced by the dideoxy termination method[66] using either Klenow (NEB) or Sequenase (USB). Both strands were sequenced. Sequence comparison to Genbank, PIR and Claverie DNA and protein databases was performed using the DFASTN and DFASTP.

protein forms channels or modifies existing channels. Alternatively, *deg-1* may encode a ligand-activated receptor. The cysteine-rich extracellular domain suggests such a function. The *u38* phenotype could then arise from an inappropriate activation of a second messenger pathway that alters the activity of membrane proteins. A further possibility is that the *deg-1(u38)* product interferes with essential cell functions, as hypothesized for the abnormal catabolism of the *β*-amyloid precursor protein in Alzheimer's disease[42].

The suppression of deaths by *mec-6* mutations indicates that an important role for *mec-6* in neurodegeneration. This *mec-6* activity could be required for the *mec-4(d)* and *deg-1* degenerations either because it activates the products of these genes (perhaps by regulating their synthesis or subcellular localization) or because it is a necessary target (such as a channel) or cofactor for their action. In all these cases, loss of *mec-6* activity would prevent the neuronal degeneration. The further molecular analysis of the *deg-1*, *mec-4* and *mec-6* genes as well as the localization of their products will be important in elucidating the ways these genes act.

The selective degeneration of particular sets of neurons is characteristic of a number of human genetic diseases[1], but the relationship between mutationally induced neurodegeneration in *C. elegans* and in humans or other organisms is unknown. Nonetheless, parallels are seen. Many late-onset human neurodegenerative diseases are expressed as dominant traits[1], indicating that they could result from the production of abnormal products. Although more dominant human genetic diseases are known than recessive or X-linked traits[43], the proportion of dominant, late-onset diseases of the nervous system seems particularly high. Another similarity between the *C. elegans* degenerations and those in human diseases may be their appearance. It is difficult to determine whether the pathologies described for the human diseases are identical to the vacuolated deaths seen in the *C. elegans* mutants. Yet in humans, vacuolated cortical cells ('ballooned neurons') occur in some patients with neurodegenerative diseases, including instances of Alzheimer's disease[44], and vacuolated cells have been reported in two cases of motor neuron disease[45,46]. Vacuolization is also seen in genetically induced neuronal death in several mouse mutants[47–49].

Although the *mec-4(d)* and *deg-1*-induced degenerations are distinct from programmed cell deaths in *C. elegans*, mechanisms similar to those occurring in these mutants may function not only in neurodegenerative diseases, but also in cell deaths that arise during normal development in other organisms. Several researchers have reported that some, but not all naturally occurring neuronal deaths in vertebrates are characterized by an initial dilation and vacuolization of the cytoplasm of the affected cells[50–53]. The process of cell death in these instances may be similar to the *C. elegans* degenerations. Such cell death could, as with the PVC cells in *deg-1(u38)* mutants, exhibit delayed onset. Such a process may occur in the death of the subplate neurons of the mammalian cerebral cortex which die with a vacuolated appearance after making functional embryonic synapses[54,55]. It seems likely that cell death during normal development occurs through more than one mechanism, one of which could utilize a *deg-1(u38)*- or *mec-4(d)*-like product. ☐

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1. Adams, R. D. & Victor, M. in *Principles of Neurology* 3rd edn Ch. 42 859–901 (McGraw-Hill, New York, 1985).
2. Hayden, M. R. *Huntington's Chorea* (Springer, Berlin, 1981).
3. Gusella, J. F. *et al. Nature* **306**, 234–238 (1983).
4. Mulder, D. W., Kurland, L. T., Offord, K. P. & Beard, M. *Neurology* **36**, 511–517 (1986).
5. Davies, P. *Neurobiol. Aging* **7**, 459–466 (1986).
6. Harding, A. E. in *Hereditary Ataxias and Related Disorders* Ch. 10, 129–165 (Churchill Livingstone, Edinburgh, 1984).

7. Chalfie, M. & Au, M. *Science* **243,** 1027–1033 (1989).
8. Chalfie, M. & Sulston, J. *Devl Biol.* **82,** 358–370 (1981).
9. Chalfie, M. *et al. J. Neurosci.* **5,** 956–964 (1985).
10. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. *Devl Biol.* **100,** 64–119 (1983).
11. Sulston, J. E. & Horvitz, H. R. *Devl Biol.* **56,** 110–156 (1977).
12. Herman, R. K. *Genetics* **116,** 377–388 (1987).
13. Way, J. C. & Chalfie, M. *Genes Dev.* **3,** 1823–1833 (1989).
14. Brenner, S. *Genetics* **77,** 71–94 (1974).
15. Greenwald, I. S. & Horvitz, H. R. *Genetics* **96,** 147–164 (1980).
16. Collins, J., Saari, B. & Anderson, P. *Nature* **328,** 726–728 (1987).
17. Suzuki, D. T. *Science* **170,** 695–706 (1970).
18. Ferguson, E. L. & Horvitz, H. R. *Genetics* **123,** 109–121 (1989).
19. Ellis, H. M. & Horvitz, H. R. *Cell* **44,** 817–829 (1986).
20. Avery, L. & Horvitz, H. R. *Cell* **51,** 1071–1078 (1987).
21. Choi, D. *Neuron* **1,** 623–634 (1988).
22. White, J. G., Albertson, D. G. & Anness, M. A. *Nature* **271,** 764–766 (1978).
23. Sulston, J. E. & Horvitz, H. R. *Devl Biol.* **82,** 41–55 (1981).
24. Coulson, A., Sulston, J., Brenner, S. & Karn, J. *Proc. natn. Acad. Sci. U.S.A.* **83,** 7821–7825 (1986).
25. Savage, C. *et al. Genes Dev.* **3,** 870–881 (1989).
26. Hubbard, S. C. & Ivatt, R. J. *A. Rev. Biochem.* **50,** 555–583 (1981).
27. Adams, G. A. & Rose, J. K. *Cell* **41,** 1007–1015 (1985).
28. Yanagi, Y. *et al. Nature* **308,** 146–149 (1984).
29. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308,** 153–158 (1984).
30. Yamamoto, T. *et al. Cell* **39,** 27–38 (1984).
31. Ullrich, A. *et al. Nature* **309,** 418–425 (1984).
32. Wen, D. *et al. Biochemistry* **26,** 4350–4357 (1987).
33. Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A. & Shooter, E. M. *Nature* **325,** 593–597 (1987).
34. Trent, C., Tsung, N. & Horvitz, H. R. *Genetics* **104,** 619–647 (1983).
35. Ferguson, E. L. & Horvitz, H. R. *Genetics* **110,** 17–72 (1985).
36. Folstein, S. E. *et al. Science* **229,** 776–779 (1985).
37. Leonard, J. P. & Salpeter, M. M. *J. Cell Biol.* **82,** 811–819 (1979).
38. Hogan, P. G. *J. Neurosci.* (in the press).
39. Choi, D. W. *J. Neurosci.* **7,** 369–379 (1988).
40. Takumi, T., Ohkubo, H. & Nakanishi, S. *Science* **242,** 1042–1045 (1988).
41. Murai, T., Kakizuka, A., Takumi, T., Ohkubo, H. & Nakanishi, S. *Biochem. biophys. Res. Commun.* **161,** 176–181 (1989).
42. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M. & Masters, C. L. *Nature* **325,** 733–736 (1987).
43. McCusick, V. A. *Mendelian Inheritance in Man* 8th edn (Johns Hopkins University Press, Baltimore, 1989).
44. Dickson, D. W. *et al. Acta neuropath.* **71,** 216–223 (1986).
45. Kohn, R. *J. Neurol. Neurosurg. Psychiat.* **34,** 427–431 (1971).
46. Reif-Kohn, R. & Mundel, G. *Confin. Neurol.* **36,** 23–32 (1974).
47. Duchen, L. W. & Strich, S. J. *J. Neurol. Neurosurg. Psychiat.* **31,** 535–542 (1968).
48. Caddy, K. W. T. & Briscoe, T. J. *Brain Res.* **91,** 276–280 (1975).
49. LaVail, M. M., Blanks, J. C. & Mullen, R. J. *J. comp. Neurol.* **212,** 217–230 (1982).
50. Pilar, G. & Landmesser, L. *J. Cell Biol.* **68,** 339–356 (1976).
51. Chu-Wang, I.-W. & Oppenheim, R. W. *J. comp. Neurol.* **177,** 33–58 (1978).
52. Knyihar, E., Csillik, B. & Rakic, P. *Science* **202,** 1206–1209 (1978).
53. Cunningham, T. J. *Int. Rev. Cytol.* **74,** 163–186 (1982).
54. Valverde, F. & Facal-Valverde, M. V. *J. comp. Neurol.* **269,** 168–192 (1988).
55. Chun, J. J. M. & Shatz, C. J. *J. comp. Neurol.* **282,** 555–569 (1989).
56. Way, J. C. & Chalfie, M. *Cell* **54,** 5–16 (1988).
57. Hedgecock, E. M., Sulston, J. E. & Thomson, J. N. *Science* **220,** 1277–1279 (1983).
58. Chalfie, M. & Thomson, J. N. *J. Cell Biol.* **82,** 278–289 (1979).
59. Ward, S., Thomson, N., White, J. G. & Brenner, S. *J. comp. Neurol.* **160,** 313–337 (1975).
60. White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. *Phil. Trans. R. Soc.* **B314,** 1–340 (1986).
61. Emmons, S. C., Klass, M. R. & Hirsh, D. *Proc. natn. Acad. Sci. U.S.A.* **76,** 1333–1337 (1979).
62. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
63. Ish-Horowicz, D. & Burke, J. F. *Nucleic Acids Res.* **9,** 2989–2998 (1981).
64. Fire, A. *EMBO J.* **5,** 2673–2680 (1986).
65. Ozkaynak, E. & Putney, S. D. *BioTechniques* **5,** 770–773 (1987).
66. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).

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LETTERS TO NATURE

Rapid recovery of the Vela pulsar from a giant glitch

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OBSERVATIONS of the rotational behaviour of pulsars have shed much light on the structure of neutron stars[1,2]. In particular, the response of the Vela pulsar (PSR0833−45) following each of seven giant glitches (sudden increases in rotation rate ν of magnitude $\Delta\nu/\nu \approx 10^{-6}$, and in the derivative, $\Delta\dot{\nu}/\dot{\nu} \approx 10^{-2}$) has been a recovery on two timescales, of about 6 and 60 days. This has been interpreted as evidence for the existence of distinct regions of neutron superfluid within the stellar crust[2]. Here I report observations that started 35 minutes after the eighth glitch of PSR0833−45, which reveal a hitherto unseen glitch component: a (12±1)% increase in the slowdown rate $\dot{\nu}$ (the largest so far seen), most of which decayed away over 0.4 days. These data support the existence of at least two superfluid components in the stellar crust that are coupled linearly to the normal crust, as suggested recently by Alpar *et al.*[3].

The Vela pulsar has been monitored nearly daily since 1984 at 1.644 and 2.3256 GHz, with the 26-m antenna at the Hartebeesthoek Radio Astronomy Observatory. During 1988, observations were made hourly to increase the chances of observing a glitch. Shortly after the pulsar rose on 24 December 1988 (JD 2447520) it spun up[4,5]. This giant glitch was detected at the next observation 35 minutes later. Continuous data capture was automatically initiated, and continued for the next 10 days, for the $11\frac{1}{2}$ hours per day that the pulsar is observable. Less frequent observations have been made since then.

Pulse arrival times were obtained from 500-pulse (45-s) on-line integrations of the pulsed signal, synchronized with the apparent pulse period, using conventional equipment and reduction methods. Time was provided by a hydrogen maser, and referred to universal time using the global positioning satellite system. Station arrival times were reduced to the Solar System barycentre and to infinite observing frequency using the Harvard-Smithsonian Center for Astrophysics PEP 740R ephemeris and reduction software (J. F. Chandler, personal communication). The optical position[6], with zero proper motion, was used. The resulting averaged arrival times had a short-term r.m.s. scatter of 10–20 μs.

The pre-glitch rotation parameters (rotation frequency ν and

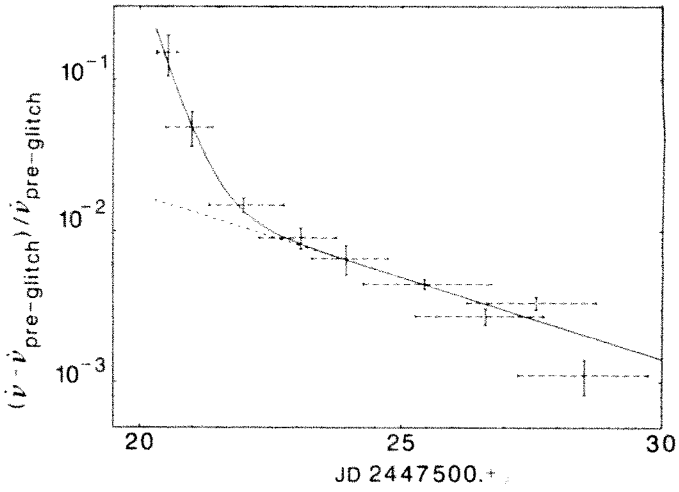

FIG. 1 Residuals in $\dot{\nu}$ for PSR0833−45 after removal of the linear and longest exponential decay terms listed in Table 1. The dashed curve is the 4-d decay from Table 1; the solid curve is the sum of the 4-d and 0.4-d decays. The dotted vertical line marks the glitch epoch. Data are normalized to the pre-glitch value of $\dot{\nu}$. Vertical error bars are ±1σ. Horizontal error bars show the range of data fitted.

TABLE 1 PSR0833 − 45 rotation parameters at the time of the 1988 glitch

Pre-glitch parameters	
ν_0	11.20001539350(2) Hz
$\dot{\nu}_0$	$155.8232(4)\times10^{-13}$ Hz s^{-1}
$\ddot{\nu}_0$	8.3(9) Hz s^{-2}
Epoch, t_0	JD 2447493.15
Range fitted	JD 2447480–2447520
RMS residual	180 μs
Glitch parameters	
$\Delta\nu/\nu$	$1.8071(8)\times10^{-6}$
$\Delta\dot{\nu}/\dot{\nu}$	$12(1)\times10^{-2}$
Epoch	JD 2447520.303(1)
Range fitted	JD 2447480–2447521.76
Post-glitch decay	
$\Delta\dot{\nu}_1/\dot{\nu}$	$20(9)\times10^{-2}$
$\Delta\dot{\nu}_2/\dot{\nu}$	$1.6(3)\times10^{-2}$
$\Delta\dot{\nu}_3/\dot{\nu}$	$0.291(6)\times10^{-2}$
τ_1	0.4(1) d
τ_2	4.0(5) d
τ_3	96(5) d
$(\dot{\nu}_0-\dot{\nu}_{\text{pre-glitch}})/\dot{\nu}$	$0.488(4)\times10^{-2}$
$\ddot{\nu}_0$	8×10^{-22} Hz s^{-2} (fixed)
Range fitted	JD 2447520.3–2447840

The numbers in parentheses are the 1σ errors quoted in the last digit.

its derivatives) and their changes at the time of the glitch ($\Delta\nu/\nu$, $\Delta\dot{\nu}/\dot{\nu}$) are listed in Table 1. The change in frequency derivative $\dot{\nu}$ decays rapidly following the glitch, so $\Delta\dot{\nu}/\dot{\nu}=(12\pm1)\%$, obtained from a fit to 1.5 days of post-glitch data that assumed constant $\ddot{\nu}$, must be regarded as a lower limit. This value of $\Delta\dot{\nu}/\dot{\nu}$ is three times any seen before in PSR0833 − 45 and twice the previous largest value, seen in PSR0355 + 54 (ref. 7). The glitch epoch agrees with that reported by Hamilton *et al.*[5], who were observing the pulsar at the time of the event.

To study the post-glitch behaviour of the pulsar, models for the recovery of $\dot{\nu}$ were fitted to 320 days of post-glitch data. Successive short spans of data were fitted for ν and $\dot{\nu}$, resulting in a set of ($\dot{\nu}_i$, t_i) values. These data were then fitted with a sum of simple exponential decays and a linear decrease in $|\dot{\nu}|$ following the glitch epoch t_g

$$\dot{\nu}(t)=\dot{\nu}_0+\ddot{\nu}_0(t-t_g)+\sum_i\Delta\dot{\nu}_i\exp\left[-(t-t_g)/\tau_i\right]$$

This method enhances the detectability of the decay in $\dot{\nu}$ above the 'timing noise', which is particularly evident in the Vela pulsar as large systematic excursions in rotation phase[8,9].

Cordes *et al.*[8] obtained good fits to data following six glitches by using two exponential decays, of characteristic timescales $\tau_i\approx6$ d and $\tau_i\approx60$ d. The 1988 glitch is best fitted with two decays with similar parameters, plus another, much shorter (0.4 ± 0.1 d), exponential term which contains most of the jump in $\dot{\nu}$ ($\Delta\dot{\nu}/\dot{\nu}=0.20\pm0.09$) (Table 1, Fig. 1). As the ($\dot{\nu}_i$, t_i) data were obtained from fits to ⩾12 hours of arrival times, the 0.4-d timescale is an upper limit. Because of the relatively short span of data, various combinations of the longest decay and linear term ($\ddot{\nu}$) could be fitted with equal success. An average value of 8×10^{-22} Hz s^{-2} was assumed for $\ddot{\nu}$ to obtain the parameters listed in Table 1.

In the 'vortex creep' model which Alpar *et al.* developed[2] and tested on the first four Vela glitches[10], and on glitches in the Crab pulsar and PSR0525 + 21 (ref. 11), the neutron star has two components: (1) The tightly coupled interior, crust and magnetosphere which constitute the bulk of the star. We observe the rotation of this component through radio emission from the magnetosphere. (2) A layer of superfluid within the crust which is relatively loosely coupled to the crust, and therefore lags the general slowdown of the star. In this model, the glitch is caused by a sudden transfer of angular momentum from part of the crustal superfluid to the rest of the star. The rotational coupling between the crustal superfluid and crust is dependent on the difference ω between the crustal and superfluid rotation rates. The superfluid thus decouples from the star during a glitch, and recouples as ω redevelops following the glitch. This is observed as an increase in $|\dot{\nu}|$ of the crust at the glitch, followed by its decay towards the pre-glitch value. An abnormally high value of $\ddot{\nu}$ is therefore also evident during the years of post-glitch recovery, and seems to persist until the next glitch.

Alpar *et al.*[2] assumed that the crustal superfluid coupling depends exponentially on ω, and thus proposed that the recovery of $\dot{\nu}$ will be delayed by $t_0=\Delta\nu/|\dot{\nu}|$, typically two to three weeks for the Vela pulsar. Because the epochs of the early Vela glitches were uncertain by one to three weeks, this prediction could not be fully tested. Signs of immediate post-glitch recoupling on timescales of 1.6 and 3.2 d were observed in two recent Vela glitches[12,13]. The first post-glitch observations of these events were 0.8 and 0.7 d after the glitches respectively, which precluded the detection of faster decays. Similar behaviour has recently been observed in PSR0355 + 54 following a spin-up[7] that had a predicted recovery delay t_0 of five years and yet recovered partially on a timescale of 44 days. This last event has been modelled[3] by including a crustal superfluid component in which the superfluid-to-crust coupling depends linearly on ω. The observations reported here of two short decays immediately following the glitch imply that at least two similar components may exist in the Vela pulsar.

The pre-glitch $\dot{\nu}$ was $-(155.804\pm0.003)\times10^{-13}$ Hz s^{-1}, which is in the range $-(156.0\pm0.3)\times10^{-13}$ Hz s^{-1} noted by Cordes *et al.*[8] for the first six Vela glitches. No sign of any glitch precursor was evident in the timing data. Over the six months before the glitch, $\ddot{\nu}$ had decreased to $(1.7\pm0.2)\times10^{-22}$ Hz s^{-2}. This is well below any value reported by Cordes *et al.*, and greater than the theoretical 'unglitched' value $\ddot{\nu}=n\dot{\nu}^2\nu^{-1}=0.7\times10^{-22}$ Hz s^{-2} with braking index $n=3$. This implies that the recovery following the previous glitch may have been nearing completion, although $\ddot{\nu}$ over such short data spans varies markedly during interglitch epochs, owing to timing noise in $\dot{\nu}$.

Timing noise was measured as the r.m.s. phase residual to a 100-d fit, after removal of the three decay components. The level of timing noise following this glitch is (60 ± 35) μs, similar to that reported by Cordes *et al.*[8] following previous glitches. Immediately after the glitch, timing noise is higher than average (~180 μs), implying that the three-component decay model may be too simple.

Following a suggestion (C. R. Gwinn, personal communication) that the disturbed star may nutate immediately following a glitch, periodic variations were searched for in the pulse shape, pulse strength and timing data of the first day following the glitch. Periodograms were constructed and evaluated according to Scargle[14]. No significant periodicities were found down to 2.6 min. The variations in timing and flux data of longer than a day were consistent with those expected from timing noise and interstellar scintillation respectively, and were not truly periodic. □

Received 17 January; accepted 10 April 1990.

1. Baym, G., Pethick, C., Pines, D. & Ruderman, M. *Nature* **224**, 872–874 (1969).
2. Alpar, M. A., Anderson, P. W., Pines, D. & Shaham, J. *Astrophys. J.* **276**, 325–334 (1984).
3. Alpar, M. A., Cheng, K. S., Pines, D. & Shaham, J. *Mon. Not. R. astr. Soc.* **233**, 25–31 (1988).
4. Flanagan, C. *IAU Circ.* No. 4695 (1988).
5. Hamilton, P. A., King, E. A., McConnell, D. & McCulloch, P. M. *IAU Circ.* No. 4708 (1989).
6. Manchester, R. N. *et al. Mon. Not. R. astr. Soc.* **184**, 159–170 (1978).
7. Lyne, A. G. *Nature* **326**, 569–571 (1987).
8. Cordes, J. M., Downs, G. S. & Krause-Polstorff, J. *Astrophys. J.* **330**, 847–869 (1988).
9. Downs, G. S. *Astrophys. J.* **249**, 687–697 (1981).
10. Alpar, M. A., Anderson, P. W., Pines, D. & Shaham, J. *Astrophys. J.* **278**, 791–805 (1984).
11. Alpar, M. A., Nandkumar, R. & Pines, D. *Astrophys. J.* **288**, 191–195 (1985).
12. McCulloch, P. M., Hamilton, P. A., Royle, G. W. R. & Manchester, R. N. *Nature* **302**, 319–321 (1983).
13. McCulloch, P. M., Klekociuk, A. R., Hamilton, P. A. & Royle, G. W. R. *Aust. J. Phys.* **40**, 725–730 (1987).
14. Scargle, J. D. *Astrophys. J.* **263**, 835–853 (1982).

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Measurement of the London moment in two high-temperature superconductors

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THE well-known London equations dictate that the magnetic field inside a stationary superconductor vanishes. The same equations, however, predict that the superconductor generates a magnetic field when it is put into uniform rotation. The associated magnetic moment is known as the London moment. Here we show that high-transition-temperature (high-T_c) superconducting oxides indeed possess this fundamental property, and when rotated, develop a London moment of the same magnitude as that of conventional superconductors, provided that the signal is scaled to the shielded volume fraction. This confirms that high-T_c superconductivity corresponds to a superfluid phase in the London sense, and that the mass of the carriers that appears in the expression of the London moment is the free-electron mass, irrespective of the nature of the carriers in the normal state.

The London field is given by[1]

$$\mathbf{B} = -\frac{2m}{e}\boldsymbol{\omega}, \qquad (1)$$

where $\boldsymbol{\omega}$ is the angular frequency, $-e$ is the electron charge and m is the mass of the charge carriers to be discussed below. The origin of the effect may be understood in several ways, the earliest argument being that of Becker *et al.*[2] Consider a charged superfluid in a matrix of positively charged ions, with no frictional coupling between these two systems. Consequently, when the superconductor is put into rotation one could initially view the superfluid as unaffected by this motion and remaining immobile. But the rotating positively charged ion matrix constitutes a current that generates a magnetic field. This generates an electric field by induction, so that the superfluid is set into rotation until the lattice and the superfluid move in unison, except for a surface layer of thickness λ where the superfluid electrons lag behind. The net effect is thus a surface current that generates the magnetic field in equation (1). Alternatively, one can derive the London moment using conservation of total angular momentum, as for other gyromagnetic phenomena[1,3]. These arguments, however, are not sufficient. Superconductivity is a macroscopic quantum phenomenon and is described by a unique ground state. As a consequence the same London moment is also developed when cooling a rotating superconductor through its transition temperature T_c. To show this we recall that the London moment can be derived from the London equation $\nabla \times \mu_0 \lambda_L^2 \mathbf{j}_s = -\mathbf{B}$, in combination with the Maxwell equation $\nabla \times \mathbf{B} = \mu_0 \mathbf{j} = \mu_0(\mathbf{j}_s - n_s e \mathbf{v}_0)$. Here $\mathbf{j}_s$ is the supercurrent density, $\lambda_L^2 = m/\mu_0 n_s e^2$ the London penetration depth, n_s the density of superfluid electrons and $\mathbf{v}_0 = \boldsymbol{\omega} \times \mathbf{r}$ the velocity of the positively charged lattice. Solving these equations for the magnetic field yields

$$\nabla^2 \mathbf{B}_{\mathrm{eff}} = \frac{1}{\lambda_L^2}\mathbf{B}_{\mathrm{eff}} \qquad (2)$$

where

$$\mathbf{B}_{\mathrm{eff}} = \mathbf{B} + 2\mu_0 n_s e \lambda_L^2 \boldsymbol{\omega} = \mathbf{B} + \frac{2m}{e}\boldsymbol{\omega} \qquad (3)$$

Equations (2) and (3) tell us that except for a surface layer of thickness λ_L the field inside a rotating superconductor is equal to $-(2m/e)\omega$, as in equation (1). Various arguments (see, for example, refs 4, 5) state that m in equation (1) should be the bare-electron mass m_e, at least if relativistic effects are disregarded[6]. In view of the debate about the nature of the carriers and of the (possibly exotic) pairing mechanisms in the high-T_c superconductors it seemed worthwhile to show that they possess the same fundamental property.

The London moment was measured on sintered $YBa_2Cu_3O_{7-\delta}$ and $BaPb_{0.8}Bi_{0.2}O_3$. For the $YBa_2Cu_3O_{7-\delta}$ sample the onset transition temperature as determined by resistance and magnetic measurements is 92.0 K. The width of the transition in the resistance measurement is 0.5 K. The sample shows full shielding as measured in susceptibility in an a.c. field of 24 μT. The Meissner effect determined using a SQUID (superconducting quantum-interference device) in a field of 0.2 mT corresponds to 25% of the total volume. For $BaPb_{0.8}Bi_{0.2}O_3$ the transition temperature determined in a.c. susceptibility is 10.0 K. The sample shows only 91% shielding at 4.2 K. The partial shielding is a problem generally encountered in the ceramic superconductors and may be related to inhomogeneous composition and weak coupling between grains.

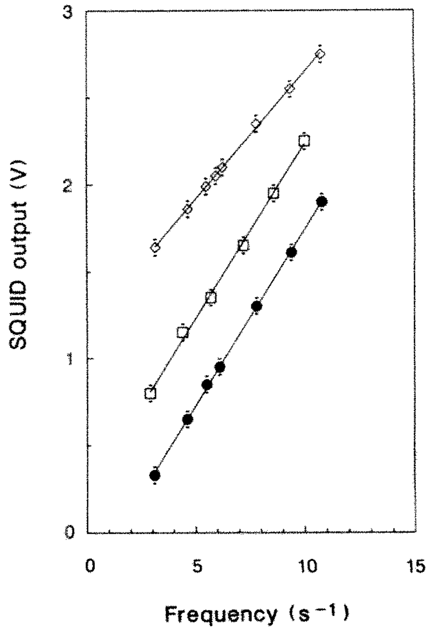

FIG. 1 The change in the SQUID output voltages as a function of the frequency of rotation for $BaPb_{0.8}Bi_{0.2}O_3$ (◇), Pb (□) and $YBa_2Cu_3O_{7-\delta}$ (●). 1 V corresponds to $(3.6 \pm 0.2) \times 10^{-10}$ T. The vertical position of the data is arbitrary, only the slope is relevant. The $YBa_2Cu_3O_{7-\delta}$ sample shows the same London moment as the Pb sample to within the experimental error, a least-squares fit to the data points gives slopes 0.202 ± 0.007 V s and 0.198 ± 0.008 V s for $YBa_2Cu_3O_{7-\delta}$ and Pb respectively. For the $BaPb_{0.8}Bi_{0.2}O_3$ sample the slope is 25% smaller due to non-ideal shielding and a slight difference in sample dimensions. Both samples were pressed into pellets of ~2-mm thickness and 12-mm diameter and sintered at the appropriate temperatures.

The London moment was measured by rotating the disk-shaped superconductors inside a flux transformer which couples the magnetic flux to a commercial r.f. SQUID. All measurements were done by first cooling to 4.2 K and then setting the sample into rotation. This results in a signal that is proportional to the shielded volume fraction in a zero-field cooled magnetization measurement. A lead sample shaped as closely as possible to the same dimensions as the $YBa_2Cu_3O_{7-\delta}$ sample is used to calibrate the measurement. When inserting the free-electron mass and charge in equation (1) a magnetic field of $\omega \times 1.14 \times 10^{-11}$ T is expected, with ω in rad s^{-1}. With the measured self-inductance of the flux transformer and an estimate of the fraction of the flux actually detected, the intensity of the magnetic field measured for the lead sample is within 10% of this value. The 10% error is the result of uncertainties in the geometrical factors and the actual self-inductance of the flux transformer with the superconductor in the primary coil. This error only affects the absolute values. For the measurement on the high-temperature superconductors the lead sample serves as a reference.

Figure 1 shows that the magnetic moment varies linearly with frequency. The moment changes sign when the sense of rotation is reversed. The sign of the signals of the three samples is the same. From Fig. 1 it is clear that the signals of $YBa_2Cu_3O_{7-\delta}$ and Pb are equal, within the experimental error. For $BaPb_{0.8}Bi_{0.2}O_3$ the signal is about 25% smaller than that of the Pb reference sample. This may be attributed to the non-ideal shielding found for the $BaPb_{0.8}Bi_{0.2}O_3$ sample, and to slightly different sample dimensions. When corrections are applied for the coupling factor of the signal to the flux transformer and the signal is scaled to the measured shielded volume fraction, it is again equal to the Pb reference signal to within the estimated accuracy. We conclude that the abundance of defects and grain boundaries in our sintered high-T_c materials have no other effect on the London moment than to reduce it in proportion to the reduced shielding.

From these observations we conclude that the superconductivity in $YBa_2Cu_3O_{7-\delta}$ and $BaPb_{0.8}Bi_{0.2}O_3$ corresponds to a superfluid phase in the London sense. The value of the London moment is equal to that of conventional BCS superconductors. From this it follows in particular that the mass in equation (1) is the free-electron mass irrespective of the material. This last result is of some interest in view of the special nature of the oxide superconductors, which can be thought of as being derived from semiconductors by suitable doping or varying the oxygen concentration (self-doping). The conductivity in the normal state is associated with negatively charged carriers in $BaPb_{0.8}Bi_{0.2}O_3$ but with positive carriers (holes) in the case of $YBa_2Cu_3O_{7-\delta}$. Because of the low carrier densities and very small coherence lengths, it has been proposed (see, for example, refs 7, 8) that real-space pairing is the superconductivity mechanism, as opposed to the **k**-space (Cooper) pairing of the BCS theory. One might expect heavy masses for such carriers but this work demonstrates that the effective-mass concept is irrelevant for the London moment, that is, it involves the bare mass m_e irrespective of the nature of the carriers in the normal state. This may be understood from the fact that the London moment is a magneto-mechanical effect[3] and therefore involves the true mechanical momentum m_ev of the carriers (see also refs 4, 5). ☐

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1. London, F. *Superfluids* Vol. 1 (Wiley, New York, 1950).
2. Becker, R., Heller, G. & Sauter, F. *Z. Physik* **85,** 772–787 (1933).
3. Broer, L. J. F. *Physica* **13,** 473–478 (1947).
4. Cabrera, B., Gutfreund, H. & Little, W. A. *Phys. Rev.* B**25,** 6644–6654 (1982).
5. Cabrera, B. & Peskin, M. E. *Phys. Rev.* B**39,** 6425–6430 (1989).
6. Tate, J., Cabrera, B., Felch, S. B. & Anderson, J. T. *Phys. Rev. Lett.* **62,** 845–848 (1989).
7. Micnas, R., Ranninger, J. & Robaszkiewicz, S. *Rev. Mod. Phys.* **62,** 113–171 (1990).
8. de Jongh, L. J. *Physica* C**152,** 171–216 (1988); *Physica* C**161,** 631–655 (1989).

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Three-dimensional twisted vortices in an excitable chemical medium

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SEVERAL different types of three-dimensional nonlinear wave structure are expected to occur in chemically excitable media[1]. In general these are vortex-like structures (Fig. 1, for example) in which the waves adopt complex spatial configurations (curved, knotted, twisted or closed into rings). But until recently only simple vortices and vortex rings have been observed experimentally, for example in the Belousov–Zhabotinsky (B–Z) reaction[2–4] and in cardiac tissue[5]. Here we report a new type of structure, twisted vortices, observed in a B–Z reaction immobilized in agarose gel (Fig. 2). In cross-section, a twisted vortex resembles a rotating spiral with a phase of rotation that changes along the filament. The filament extends through the entire medium, terminating at its free surfaces. The wavelength of a twisted vortex is shorter than that of a simple one and decreases with increasing twist, in accord with theory[6,7]. The period shows little dependence on twist, contrary to the results of computer experiments[6]. Twisted vortices decay into simple ones; their twist decays exponentially with a time constant that increases with filament length. These dynamics are consistent with the theory of diffusional untwisting[8,9] rather than that of shock waves[10].

In a three-dimensional excitable medium, simple vortices are formed readily (Fig. 1*a*). These are rotating spiral waves that represent a straightforward extension of those seen in two dimensions, the rotation phase being independent of the third coordinate. Most of the spiral waves observed experimentally (for example, in the B–Z reaction[2–4,11,12], in cardiac muscle[13], in the retina[14] and in slime molds[15]), are such simple three-dimensional vortices. But numerical experiments with a Fitz-Hugh–Nagumo model[16] and analytical theory[6] predict that twisted vortices should have smaller periods and wavelengths than other types of autonomous waves and thus should dominate over these by suppressing and expelling them.

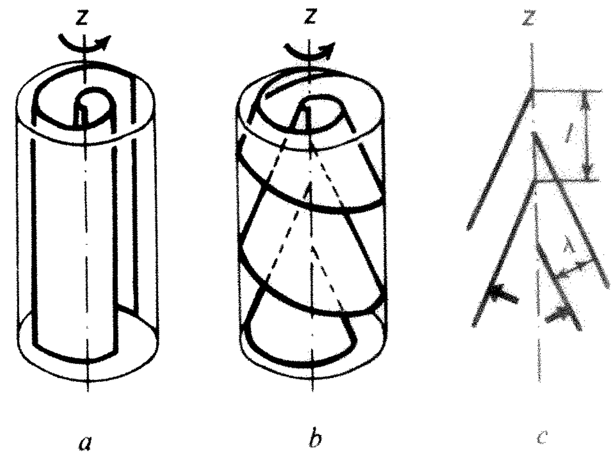

FIG. 1 Schematic representations of simple (*a*) and twisted (*b*, *c*) vortices. The top view of a twisted vortex is a rotating spiral wave. The side view comprises overlapping cones, seen in cross-section in *c*. The dash-dotted line is the rotation axis, and solid lines represent wave fronts. The directions of wave propagation and rotation are indicated by arrows. λ is the wavelength and *l* is the distance at which the phase shift is 2π. The twist $w = 2\pi/l$.

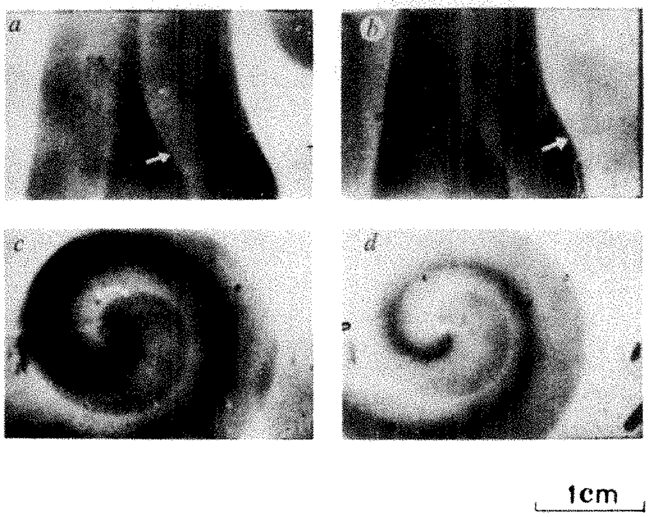

FIG. 2 A twisted vortex ($w = 3\,cm^{-1}$) in a chemically excitable medium. *a*, *b*, Side views (compare Fig. 1*c*); *c*, *d*, top views (compare Fig. 1*b*). The interval between *a* and *b*, and *c* and *d*, is 4 min. Arrows show the direction of wave propagation. Light is absorbed mostly by the portions of waves that lie perpendicular to the plane of the figure, so that in *a* and *b* only the central cross-section of the twisted vortex (Fig. 1*c*) is seen. The excitable medium is a B-Z reagent immobilized in agarose gel[4,17,18]. The recipe was chosen to make photography of the thick layers possible: (50 mM $NaBrO_3$, 50 mM $CH_2(COOH)_2$, 150 mM H_2SO_4, 0.65 mM ferroin, 0.75% agarose.) The temperature is 20 °C.

We obtained twisted vortices from simple ones by creating temperature gradients along vortex filaments. Simple vortices with straight-line filaments were obtained by the standard method[16]. In the middle of a rectangular glass cuvette containing immobilized B-Z reagent, we placed a vertical plastic screen 0.1 mm thick, thus dividing the cuvette into two compartments. In one of these, a cylindrical wave was initiated by inserting a vertical thin silver wire. On reaching the screen, the wave broke along the generatrix (a line whose motion generates a surface) of the cylinder, the breaking edges corresponding to two straight lines which moved away from each other along the screen surface. When one of these reached the wall of the cuvette, the screen was removed and the other breaking edge curled into a simple scroll with a straight-line filament. A glass top was placed on the cuvette, so that both ends of the filament were in contact with glass, to avoid inhibition of the reaction by oxygen.

To obtain a twisted scroll the cuvette was placed on a thermostat-controlled stage heated above room temperature. This caused a local increase in the reaction rate and in the velocity of vortex rotation. An increase in temperature by 1 °C caused a decrease in the period from about 10 min to 0.5 min. These differences in local rotation velocities resulted in the development of a twist. For unequal angular frequencies of rotation, ω, the phase difference $\Delta\phi$ between two cross-sections perpendicular to the vertical (z-) axis grows linearly with time ($\Delta\phi = t\Delta\omega$), so vortices with different twists w ($w = d\phi/dz$) could be obtained by varying the time and heating temperature.

Figure 2 shows a twisted vortex. The top view corresponds to a rotating spiral wave, and the side view shows cones produced by the wave fronts. As the vortex rotates, the waves move from the centre (indicated by the arrow) to the periphery at a constant velocity.

We have studied the dependence of vortex characteristics on twist. The average twist w is defined as $w = 2\pi/l$ (see Fig. 1*c*); l was measured from the photographs. An increase in w causes a decrease in wavelength λ (Fig. 3*c*) and a weaker decrease in the rotation period (Fig. 3*b*). These are consequences of the dispersion relation for the B-Z reaction. Our measurements of the dispersion curve showed that a twist-induced change in wavelength from 0.5 to 0.9 cm (as in Fig. 3*c*) corresponds to a slow change in rotation period from 9.0 to 10.5 min (similar to Fig. 3*b*).

When the twist exceeds a critical value w_c ($\approx$7–9 cm^{-1}), the regular rotation of the twisted vortex is disturbed and breaks in the wave occur (arrow in Fig. 3*a*). Further increases in w give rise to a pattern resembling turbulence[19]. There exists a minimum wavelength for a stable wave, λ_{min}, which can be estimated from the dispersion relation ($\lambda_{min} \approx 0.45$ cm); λ_c is found to be close to this value.

In theory, vortices with twists below the critical value should evolve into simple ones. Spontaneous untwisting of a twisted vortex was first demonstrated in numerical experiments[7]. Analytical theories[8-10], however, predict different untwisting kinetics. The theory of shock waves, based on the first-order differential equation $w_t = \omega_w w_z$, states that the wave of untwisting propagates with velocity ω_w along the twisted vortex[10]. A more accurate equation describing the evolution of a

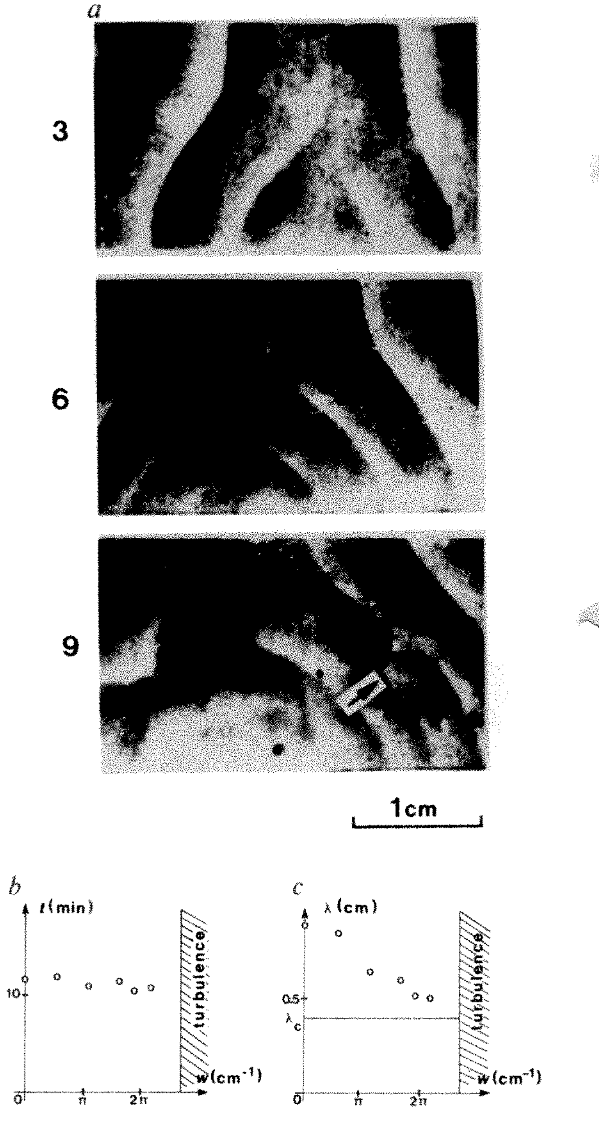

FIG. 3 Twisted vortices with different twists. *a*, Side views (compare Fig. 2*a*, *b*). Numbers on the left denote the average twist w in cm^{-1}. At $w = 9\,cm^{-1}$ a wave break is seen (arrow). *b*, *c*, Dependence of rotation period T and wavelength λ on twist w. T and λ were measured when the maximum temperature variation within the cuvette (measured with MT54M microthermistors) was no greater than 0.15 °C. The errors in the vortex rotation periods due to residual temperature gradients do not exceed 1%.

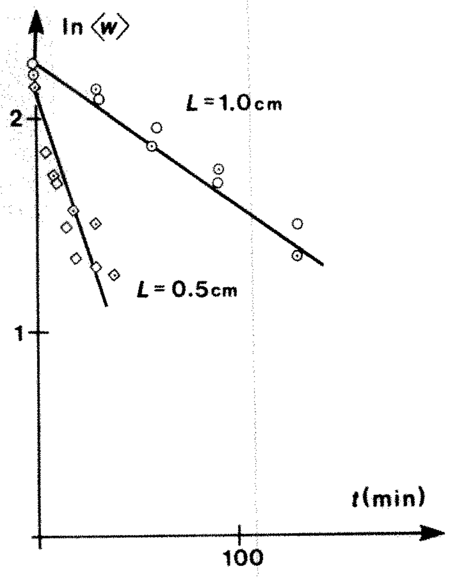

FIG. 4 Evolution of twist w. For sample thicknesses $L = 1.0$ and 0.5 cm, the kinetics of untwisting are seen to be exponential. Two sets of data are shown for each thickness. A least-squares fitting of the straight lines gives time constants τ as 161 min and 45 min respectively.

twist was proposed in ref. 8:

$$w_t = \omega_w w_z + D w_{zz} \qquad (1)$$

Solution of this equation for $\omega_w =$ constant and boundary conditions $W_z|_{z=0,L} = 0$ (where L is the cuvette length) predicts exponential untwisting:

$$w(t) \propto \exp(-t/\tau);\ \tau = (D\pi^2/L^2 + \omega_w^2/4D)^{-1} \qquad (2)$$

Our experiments confirm that vortices with twists below the critical value transform to simple ones. Moreover, the kinetics of untwisting were found to be exponential (Fig. 4) and at variance with the simple shock-wave theory of ref. 10; the time constant τ increases with L.

Equation (2) fits our experiments qualitatively but not quantitatively. Equation (2) predicts that τ is proportional to L^2 at small L. We found that a twofold increase in L (from $L = 0.5$ cm to 1 cm) indeed resulted in about a fourfold increase in τ (see Fig. 4 legend), but further increase in L (from 1 cm to 2 cm) increased τ by about a factor of seven. This remains to be explained theoretically. □

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1. Winfree, A. T. & Strogatz, S. H. *Nature* **311**, 611–615 (1984).
2. Winfree, A. T. *Science* **181**, 937–939 (1973).
3. Welsh, B., Gomatam, S. & Burgess, A. *Nature* **304**, 611–614 (1983).
4. Jahnke, W., Henze, C. & Winfree, A. *Nature* **336**, 662–665 (1988).
5. Medvinsky, A. B., Panfilov, A. V. & Pertsov, A. M. in *Self-Organization. Autowaves and Structures Far from Equilibrium* (ed. Krinsky, V.) 195–199 (Springer, Berlin, 1984).
6. Mikhailov, A. S., Panfilov, A. V. & Rudenko, A. N. *Phys. Lett.* A**109**, 246–250 (1985).
7. Panfilov, A. V., Rudenko, A. N. & Pertsov, A. M. *Dokl. Akad. Nauk SSSR* **279**, 1000–1002 (1984).
8. Biktashev, V. N. *Physica* D**36**, 167–172 (1989).
9. Keener, J. P. *Physica* D**31**, 269–276 (1988).
10. Braznik, P. K., Davydov, V. A., Zykov, V. S. & Mikhailov, A. S. *Abstr. 2nd All-Union Conference on Mathematical and Computational Methods* 118–119 (ONTI NCBI, Pushchino, 1987).
11. Zhabotinsky, A. M. & Zaikin, A. N. in *Oscillatory Processes in Biological and Chemical Systems* Vol. 2, 279–283 (ONTI NCBI, Pushchino, 1971).
12. Agladze, K. I. & Krinsky, V. I. *Nature* **296**, 424–426 (1982).
13. Allessie, M. A., Bonke, F. I. M. & Schopman, F. J. G. *Circul. Res.* **33**, 54–62 (1973).
14. Bures, J. & Gorelova, N. A. *J. Neurobiol.* **14**, 353–363 (1983).
[illegible] Gerish, G. *Curr. Topics devl. Biol.* **3**, 157 (1968).
16. Panfilov, A. V. & Pertsov, A. M. *Dokl. Akad. Nauk SSSR* **274**, 1000–1002 (1984).
17. Kuhnert, L. *Naturwissenschaften* **70**, 464–466 (1983).
18. Nosztczius, Z., Hoprthemke, W., McCormick, W. D., Swinney, H. L. & Tam, W. Y. *Nature* **329**, 619–620 (1987).
19. Agladze, K. I., Krinsky, V. I. & Pertsov, A. M. *Nature* **308**, 834–835 (1984).

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Microbially mediated cerium oxidation in sea water

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REDOX processes influence the geochemistry of many elements in the ocean, but attributing the distribution of these elements in the water column and sediments to specific redox processes is difficult because they are also influenced by non-redox processes and by other inputs that are poorly constrained. Cerium provides an opportunity to study redox processes in the ocean[1–5] because its redox chemistry leads to its enrichment or depletion ('cerium anomalies') with respect to its lanthanide neighbours. A detailed understanding of Ce geochemistry is lacking, however, because of the paucity of knowledge of the redox rates and mechanisms in natural waters. Here I report measurements of Ce(III) oxidation rates using radiotracers in seawater samples collected in the Sargasso Sea and in Vineyard Sound, Massachussetts, which are much faster than previous, indirect estimates[1]. The data indicate that the negative Ce anomaly in sea water is the result of microbial oxidation followed by preferential scavenging of Ce(IV); no abiotic oxidation was detectable. This suggests that inhibition by sunlight of microbial oxidation and scavenging of Ce and Mn contribute to the pronounced surface maxima observed for these elements.

Current knowledge of cerium redox chemistry in natural waters has been inferred from its distribution in the water column and various mineral phases, and from thermodynamic data in the literature[1–5]. These indicate that Ce is preferentially removed from oxic waters by oxidation to insoluble Ce(IV), the thermodynamically stable form. Manganese oxide phases, such as Mn nodules, are strongly enriched in Ce (refs 2, 6), indicating that oxidation on Mn oxides may be a mechanism for Ce removal. The similarity of Ce and Mn marine geochemistry is also consistent with this hypothesis. But it is not clear if this similarity arises because of Ce oxidation on Mn oxides or because the distributions of both elements are controlled by common processes. Existing data provide no information on the timescales of the redox transformations of Ce, essential for complete understanding of its geochemistry and usefulness as a redox tracer.

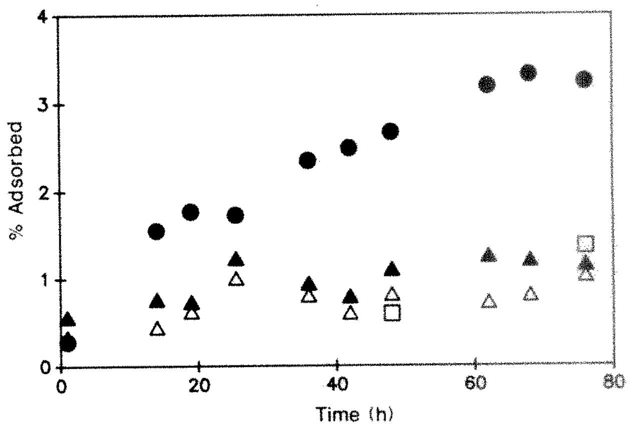

FIG. 1 Fraction of added radiotracer adsorbed on 0.2-μm-filterable particles for a sample collected at 120 m in the Sargasso Sea (32°16′ N, 64°36′ W). Samples were collected in Teflon-coated bottles on Kevlar wire and processed within 2 h of collection. All samples were incubated in the dark, at 25 °C in 2-l polycarbonate bottles. Adsorption of tracers on container walls was negligible. Filters counted in NaI detector. Absolute precision = ± 0.2%. ●, ^{139}Ce; ▲, ^{139}Ce + 1 g l^{-1} azide (added to entire sample 1 h before tracer addition); □, ^{139}Ce + 10^{-4} M ascorbate (ascorbate solution, pH 8, added to each aliquot 1 h before filtration); △, ^{152}Eu.

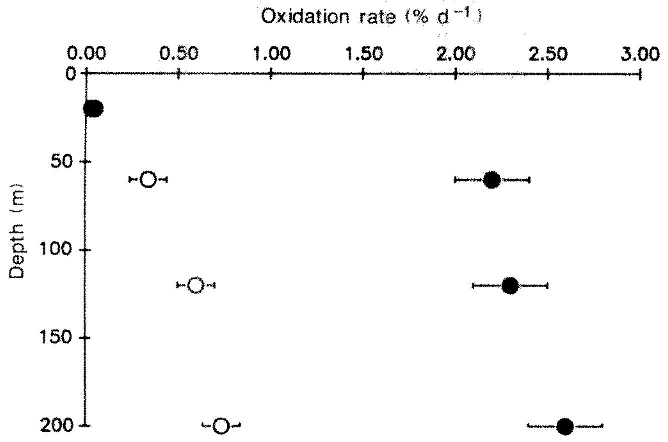

FIG. 2 Specific oxidation rates (% d^{-1}) of Ce(III) (○) and Mn(II) (●) as a function of sample depth. Calculated by subtracting ascorbate-treated samples from non-treated samples.

I have measured Ce oxidation in two diverse marine locations. Oxidation rates were measured by adding ^{139}Ce at tracer levels (that is, [^{139}Ce] < [total Ce]) to freshly collected sea water, and by following its uptake onto filterable particles. At specified times, aliquots of solution were filtered through 0.2-μm filters and the γ-activity counted. ^{152}Eu was also added as a non-redox-reactive lanthanide control to distinguish Ce oxidation from Ce(III) adsorption. Eu(III) is, however, in the middle of the lanthanide series and may be less particle reactive than Ce (III) in sea water because of stronger carbonate complexation[1,7], so an independent method was required to distinguish between Ce(III) and Ce(IV). I established that ascorbate will reduce Ce(IV) to Ce(III) in sea water at ambient pH (unpublished data). Addition of 10^{-4}M ascorbate to these samples resulted in a decrease of particulate ^{139}Ce through reduction and solubilization of Ce(IV). Because ascorbate is not a chelator and the pH is unchanged, Ce(III) is unaffected. Therefore, Ce(IV) was determined as the difference in particulate ^{139}Ce in the presence and absence of ascorbate. ^{152}Eu was not affected by ascorbate addition in Sargasso Sea samples. Ascorbate has been used in the same way to distinguish Mn oxides from particulate Mn(II) (ref. 8). Figure 1 shows results for a sample collected from 120 m at the Sargasso Sea station. Uptake of ^{139}Ce and ^{152}Eu was rapid in the first hour. Thereafter, Eu uptake was slow, approaching a steady-state value for the rest of the experiment. The uptake of ^{139}Ce continued to increase roughly linearly throughout the experiment, although at a slower rate than the initial uptake. When ascorbate was added to some aliquots and allowed to react for one hour before filtration in these aliquots, most of the particulate ^{139}Ce was re-dissolved and the remaining particulate fraction was comparable to ^{152}Eu. Therefore Ce(III) and Eu(III) uptake were comparable, and the preferential Ce uptake was the result of Ce(III) oxidation.

The oxidation of Mn(II) is microbially mediated in sea water, and the process is inhibited in the presence of azide[8,9]. Sodium azide (1 g l^{-1}) was added to samples from 120 m and incubations performed in parallel. With this treatment, uptake was comparable to that for ^{152}Eu and ^{139}Ce in the presence of ascorbate, indicating that no oxidation occurred (Fig. 1). Therefore the Ce(III) oxidation was microbial and not due to abiotic oxidation. Specific oxidation rates of Ce(III) (in % per day) as a function of depth are shown in Fig. 2, along with Mn(II) oxidation rates, which were measured using ^{54}Mn. Rates were very slow at 20 m but increased dramatically below the mixed-layer depth (25 m). The trend with depth was the same for Ce and Mn, with rates 3-4 times greater for Mn. The Mn results are in good agreement with an earlier study in the southwestern Sargasso[8].

Uptake of ^{139}Ce and ^{152}Eu in Vineyard Sound is shown in Fig. 3. Only about half of the particulate Ce that can be redissolved by ascorbate was inhibited by azide addition (Fig. 3*a*), which might indicate that the remaining fraction was oxidized abiotically. But the Eu data (Fig. 3*b*) suggests an alternative explanation. Uptake of Ce and Eu is similar for both elements and the fraction of adsorbed ^{152}Eu redissolved after ascorbate addition was equivalent to the fraction of ^{139}Ce redissolved that is not affected by azide. Therefore this fraction was probably Ce(III) and Eu(III) adsorbed on Mn oxides, which were dissolved by ascorbate, rather than Ce(IV). The concentration of particulate Mn at the beginning of the experiments was 18 nM in Vineyard Sound (compared with <1 nM in Sargasso samples), which was evidently sufficient for trivalent lanthanide adsorption to be significant. These data provide evidence that abiotic oxidation on Mn oxides is unimportant relative to microbial oxidation, even when they adsorb a significant fraction of Ce(III).

The Mn oxidation rate in Vineyard Sound was 3.9% h^{-1}. The microbial Ce oxidation rate (obtained by subtracting the uptake curve for the azide-containing solution from the azide-free solution in Fig. 3*a* and measuring the initial slope) was 1.3% h^{-1}. Therefore, microbial Mn oxidation was three times faster than the microbial Ce oxidation, similar to the Sargasso Sea results. That oxidation rates for Ce(III) and Mn(II) are within a factor of three is remarkable given the completely different speciation of each element in sea water—Mn(II) exists predominantly as $\mathrm{Mn}^{2+}_{\mathrm{aq}}$ and Ce(III) as strong carbonate complexes[7,10]. Furthermore, comparable ratios of Mn to Ce oxidation were found in both locations, spanning a 100-fold range in absolute rates. This indicates that the same microbial mechanism is involved throughout the environments studied. The close and constant relationship between oxidative removal pathways for Ce and Mn in diverse marine environments such as those studied here undoubtedly contributes to the similarities in the elements' geochemistries. No data were obtained from deep or bottom waters, but microbial Mn oxidation occurs throughout the water column, so it is likely that Ce oxidation also does.

Many trace elements are highly depleted in surface waters, but depth profiles of Ce and Mn frequently display sharp surface concentration maxima, with levels declining rapidly below the mixed layer[1,11-15]. For Mn this has been attributed to aeolian deposition[11,12] and/or photoreductive dissolution of Mn oxides in surface waters and photo-inhibition of microbial Mn oxidation (leading to the accumulation of Mn(II) in the upper photic zone[8]). The relative importance of atmospheric deposition to the *in situ* redox processes is not clear. Some elements that have no redox chemistry (such as aluminium) can also display surface maxima[16]. Under certain well stratified conditions the residence time of thorium in the upper photic zone (0-50 m) is much greater than in the lower 50-100 m. As Th has no redox chemistry, this has been attributed to differences in particle flux resulting from differences in the plankton community[17]. Therefore, the Mn maxima could arise from these processes and not redox reactions. But Ce frequently displays unique maxima not observed for the trivalent lanthanides, and it is not preferentially enriched in aeolian dust[18,19] so aeolian deposition cannot account for the maxima. Similarly, non-redox processes shown to be important for Th can also be ruled out because they would presumably lead to maxima for the other lanthanides. The unique maxima for Ce must involve its redox chemistry. At the Sargasso Sea station, low Ce oxidation rates measured in the upper photic zone would lead to a much longer residence time than in the lower photic zone, where oxidation rates are 30 times greater. This difference could lead to surface maxima. These data do not explain why oxidation at 20 m is so much slower than it is lower in the photic zone, but earlier work[8] indicates that microbial Mn oxidation is photo-inhibited, which is probably the case for Ce also. Photoreduction of Ce(IV) may also be a factor, but this has not been investigated in this study.

Given the apparent similarities between Ce and Mn, it can be argued that *in situ* redox chemistry must be of prime importance in contributing to surface Mn maxima.

Cerium oxidation is faster in Vineyard Sound than in the Sargasso Sea, but it is relatively less important than Ce(III) uptake. After 40 h, 70% of the ^{139}Ce uptake is the result of Ce oxidation in the Sargasso Sea at 120 m but only 30% in Vineyard Sound. Furthermore, because Ce(III) uptake occurs on a shorter timescale than Ce(III) oxidation, oxidation is relatively less important in environments where particle residence times are short. Therefore, Ce anomalies should be less pronounced in areas of high particle flux than low flux, and this is generally the case. Estuarine and coastal regions have little or no Ce anomaly, whereas oligotrophic waters have large negative Ce anomalies[1,4,14,15]. Adsorption kinetic data obtained from radiotracer experiments may be incorporated into non-equilibrium models of trace-element scavenging. For many elements (such as thorium, tin, scandium and zinc) this is difficult because of complex adsorption kinetics[20]. As the Ce anomaly seems to arise from a unique process with relatively straightforward kinetics, development of a Ce scavenging model may be a useful starting point for extension to more-complicated systems. Such a model would also allow more accurate predictions of the response of the Ce anomaly to changes in oceanic redox conditions. ☐

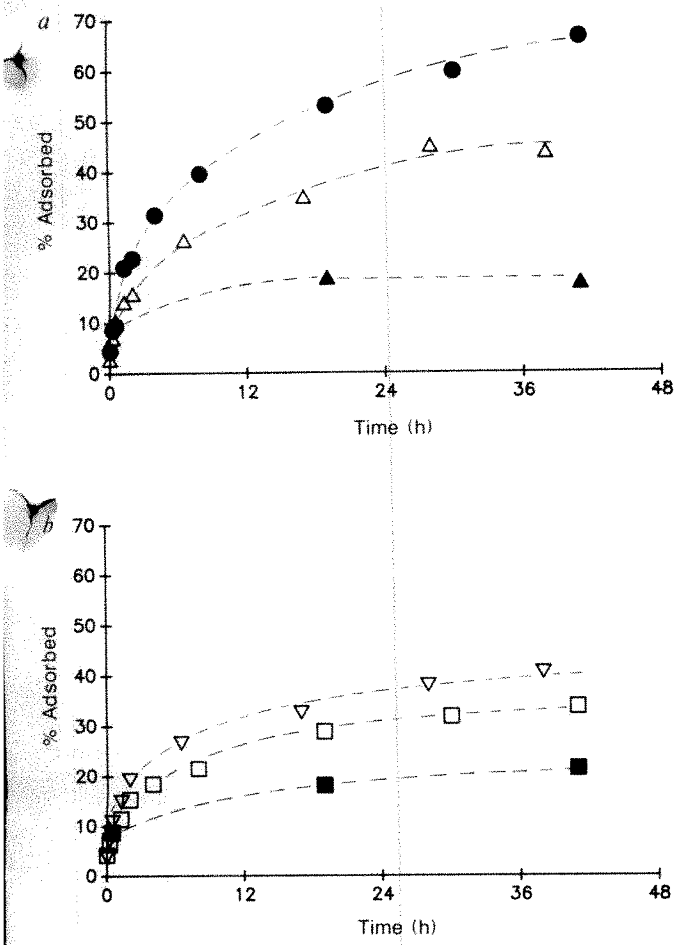

FIG. 3 Fraction of added radiotracers adsorbed on 0.2-μm-filterable particles in Vineyard Sound samples. Samples collected from a small boat in a Pyrex carboy at a location 7 km southeast of Woods Hole (10 August 1988). Samples were treated as for Sargasso samples. *a*, ●, ^{139}Ce; △, ^{139}Ce + azide; ▲, ^{139}Ce + ascorbate. *b*, □, ^{152}Eu; ▽, ^{152}Eu + azide; ■, ^{152}Eu + ascorbate. Absolute precision = ±5%. Eu uptake was significantly higher in azide-treated samples than in non-treated samples. This may be the result of alteration of particulate matter by azide or may reflect real differences between samples. Differences are small relative to other differences in this figure.

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1. Elderfield, H. *Phil. Trans. R. Soc. Lond.* A**325**, 105–126 (1988).
2. Goldberg, E. D., Koide, M., Schmitt, R. A. & Smith, R. H. *J. geophys. Res.* **68**, 4209–4217 (1963).
3. Elderfield, H. & Pagett, R. *Sci. Tot. Environ.* **49**, 175–197 (1986).
4. Sholkovitz, E. R. & Elderfield, H. *Global Biogeochem. Cycles* **2**, 157–176 (1988).
5. Murphy, K. & Dymond, J. *Nature* **307**, 444–447 (1984).
6. Elderfield, H., Hawkesworth, C. J., Greaves, M. J. & Calvert, S. E. *Geochim. cosmochim. Acta* **45**, 1231–1234 (1984).
7. Cantrell, K. J. & Byrne, R. H. *Geochim. cosmochim. Acta* **51**, 597–605 (1987).
8. Sunda, W. G. & Huntsman, S. A. *Deep Sea Res.* **35**, 1297–1317 (1988).
9. Tebo, B. M. & Emerson, S. *Biogeochem.* **2**, 149–161 (1982).
10. Turner, D. R., Whitfield, M. & Dickson, A. G. *Geochim. cosmochim. Acta* **45**, 855–882 (1981).
11. Landing, W. & Bruland, K. W. *Geochim. cosmochim. Acta* **51**, 29–43 (1987).
12. Martin, J. H. & Knauer, G. A. *Earth planet Sci. Lett.* **51**, 266–274 (1980).
13. De Baar, H. J. W., Bacon, M. P. & Brewer, P. G. *Nature* **301**, 203–204 (1983).
14. De Baar, H. J. W., Bacon, M. P., Brewer, P. G. & Bruland, K. W. *Geochim. cosmochim. Acta* **49**, 1949–1953 (1985).
15. German, C. thesis, Cambridge Univ. (1988).
16. Orians, K. J. & Bruland, K. W. *Nature* **316**, 427–429 (1984).
17. Coale, K. H. & Bruland, K. W. *Limnol. Oceanogr.* **32**, 189–200 (1987).
18. Rahn, K. A. *The Chemical Composition of Atmospheric Aerosol*, Tech. Rep. 265 (Univ. of Rhode Island, 1976).
19. Schneider, B. *J. geophys. Res.* **90**, 10744–10746 (1985).
20. Jannasch, H. W., Honeyman, B. D., Balistrieri, L. S. & Murray, J. W. *Geochim. cosmochim. Acta* **52**, 567–577 (1988).

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Ocean-ridge basalt compositions correlated with palaeobathymetry

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KLEIN and Langmuir[1] investigated the relationships between the chemistry and water depth of eruption of zero-age mid-ocean-ridge basalts around the world. They showed that the regionally averaged values of the major-element oxides Na_2O and FeO, corrected for low-pressure fractionation, and the ratio CaO/Al_2O_3, correlate with their axial depths. Klein and Langmuir pointed out that the same principles should also apply to older ocean crust, where the relationships that they had established could be used as 'calibration curves', and that there was a "possibility of petrologically constraining the origins of bathymetric anomalies throughout the ocean basins"[1]. Keen[2] showed that this can be achieved if the initial depths of eruption of older basalts are found by correcting their present depths below sea level for sediment loading and thermal subsidence. Here we show that the chemistry of basalts from older oceanic crust in the Atlantic and Indian Oceans correlates with their depth restored in this way to the original water depth at the time of eruption.

The Ocean Drilling Program's Hole 647A in the extinct mid-ocean ridge of the Labrador Sea illustrates our approach (Figs 1 and 2). Basalts were encountered at 4,561 m below sea level, beneath 699 m of sediment[3,4]. They are typical mid-ocean-ridge basalts (MORB), 56 Myr old, but still fresh[5]. Values of Na_2O and FeO corrected for low-pressure fractionation to a common MgO value of 8 wt%, $Na_{8.0}$ and $Fe_{8.0}$ respectively, and the ratio CaO/Al_2O_3, have been calculated for each of the analyses[5] that meets the criteria established by Klein and Langmuir[1] in terms of the range of acceptable values of MgO wt% (Table 1).

We found the initial depth of eruption of the basalts by 'back-tracking'[6]. Sediments have depressed the ocean crust at site 647A, and so the basalts have been 'unloaded' assuming point-wise Airy isostasy[7], using a mean value of sediment density calculated from the set of measurements for the site[3] (Table 1). The ocean crust at site 647A has also subsided thermally, and this subsidence has also been restored. We assume that the average depth at which ocean crust is formed is 2,500 m (ref. 8).

TABLE 1 Sites on mid-ocean ridges

(1) Site	(2) Lat. (deg)	(3) Long. (deg)	(4) Depth (m)	(5) Sediment (m)	(6) Igneous section (m)	(7) Basalt age (Ma)	(8) Depth to basalts: Restored (m)	(9) Depth to basalts: Unloaded (m)	(10) Depth to basalts: Now (m)	(11) Source glass rock	(12) $Na_{8.0}$	(13) $Fe_{8.0}$	(14) Number of Na, Fe analyses	(15) CaO/ Al_2O_3	(16) Number of Ca/Al analyses	(17) Refs
14	28.33 S	20.94 W	4,346	107	1.5	39	2,229	4,415	4,453	SG	—	—	—	0.740	1	20
15	30.89 S	17.98 W	3,938	141	1.4	21	2,465	4,031	4,079	SG	2.30	8.92	1	0.837	1	20
18	27.99 S	08.01 W	4,022	178	0.3	26	2,363	4,148	4,200	SG	—	—	—	0.777	1	20
19	28.54 S	23.68 W	4,685	141	4.3	51	2,284	4,783	4,826	SG	2.20	9.50	1	0.826	1	20
105	34.90 N	69.17 W	5,245	623	9	156	2,044	5,676	5,875	SG	2.06	10.45	1	0.804	1	21–24
335	37.30 N	35.20 W	3,188	454	108	13	2,244	3,506	3,642	SG	2.40	9.45	1	0.761	1	24–27
350	67.06 N	08.29 W	1,275	362	18	41	−668	1,566	1,637	DR	1.89 ± 0.04	9.64 ± 0.12	3	0.767 ± 0.010	3	28
407	63.94 N	30.58 W	2,472	304	155	35.5	629	2,714	2,776	SG	1.78	10.25	1	0.817	1	24, 29
409	62.62 N	25.95 W	832	80	239	2.4	352	894	912	DG	1.63 ± 0.20	9.17 ± 0.09	2	0.835 ± 0.023	2	24, 29
417D	25.11 N	68.04 W	5,482	343	366	118	2,320	5,733	5,830	SG	2.09 ± 0.10	9.92 ± 0.32	22	0.825 ± 0.016	22	30, 36
418A	25.04 N	68.06 W	5,511	324	544	118	Combine 417 and 418									30, 36
558	37.77 N	37.34 W	3,754	408	154	37	1,873	4,002	4,162	SG	2.27 ± 0.13	9.67 ± 0.05	2	0.755 ± 0.016	3	31
559	35.12 N	40.92 W	3,754	238	63	35	1,857	3,927	3,992	SG	2.43	9.62	1	0.725	1	31
563	33.64 N	43.77 W	3,786	365	19	35.5	1,948	4,033	4,151	SG	2.16 ± 0.01	10.02 ± 0.18	3	0.833 ± 0.027	3	31
564	33.74 N	43.77 W	3,820	284	81	35.5	1,940	4,025	4,104	SG	2.17	10.06	1	0.773	1	31
647A	53.33 N	45.26 W	3,862	699	37	56	1,692	4,311	4,561	DR	1.93 ± 0.18	11.14 ± 0.69	9	0.828 ± 0.033	14	3–5
765D	15.98 S	117.58 E	5,724	924	271	139	2,720	6,270	6,648	DR	2.05 ± 0.23	9.24 ± 1.10	10	0.805 ± 0.030	10	32, 33
All	23.42 N	52.03 W	5,615	<100	D	47	3,216	5,615	5,615	SG	2.57	8.52	1	0.691 ± 0.038	2	34, 35

(1) Data. Rationale: Mid-Atlantic Ridge and its satellites—a coherent body of data; older than 20 Myr so that subsidence would be significant; 335, from our initial pilot study; 765D (Indian Ocean), deep now; All, deep formerly; 350, 407 and 409, shallow now and formerly. The data shown here form the complete Smithsonian data base for Atlantic glasses older than 20 Myr, supplemented by whole-rock data and DSDP glass data for extremes in depth. No data analysed have been excluded. (6) Section drilled through igneous rocks. D, dredge haul. (7) Age: ref. 36 throughout. (8) and (9), Density: sea water, 1.03 g cm^{-3}; mantle, 3.33 g cm^{-3}. (11) Source: D, Deep Sea Drilling Project/Ocean Drilling Program volumes; S, Smithsonian Volcanic Glass Project Data; G, glass; R, whole rock. (12)–(16) Criteria for inclusion in Table 1 and in plots. All data: meet MgO criteria of ref. 1. Smithsonian Volcanic Glass Project Data: inclusion in that data base. Glass analyses, DSDP: 409: ref. 37, Table 3, samples 409 7 6b, 409 13 2; both glasses; K_2O mean 0.15. We observe that the average of the two totals is 97.80. Whole-rock analyses: DSDP/ODP: 350: ref. 28, p. 661, Table 3, three analyses: H_2O (total) average 1.16; K_2O average, 0.28; total average, 99.27. 647A: ref. 5, Table 1: analyses for Site 647A: total average, 99.38; K_2O average, 0.05; average $H_2O+1.38$ (of eight analyses reporting). 765D: ref. 32, analyst T. Plank: Table 765-P-2, analyses with $0.0 < \text{LOI} < 0.6$; these had: total average, 99.52; K_2O average, 0.50; LOI average, 0.33. LOI is loss on ignition. (12), (13) and (15), Fig. 3: correction for low-pressure fractionation: see refs 1, 12: $Na_{8.0} = Na_2O + 0.373 \times (MgO) - 2.98$; $Fe_{8.0} = FeO + 1.664 \times (MgO) - 13.313$. $Na_{8.0}$ and $Fe_{8.0}$ are calculated for samples with 5.0–8.5% MgO. CaO/Al_2O_3 are calculated for samples with >5.0% MgO. $Fe^{G}_{8.0} = 2.033 \times (5.233 - b_{fe})$, where $b_{fe} = Na_{8.0} - 0.19 \times Fe_{8.0}$. (14) and (16), Numbers of analyses: numbers of analyses available that passed screening.

After 56 Myr, crust formed at this water depth should have subsided to 5,119 m (Table 1). But the depth of the basalts at site 647A corrected for unloading (4,311 m) is 808 m less than this. Assuming that initial differences in elevation are preserved as the crust subsides overall, the initial depth of the crust at site 647A becomes 1,692 m, not 2,500 m. This value is consistent with the generally shallow crust in the Labrador Sea, attributed to the effects of a hotspot that developed about 60 Myr ago[4,9-11] beneath the Davis Strait to the north or beneath eastern Greenland.

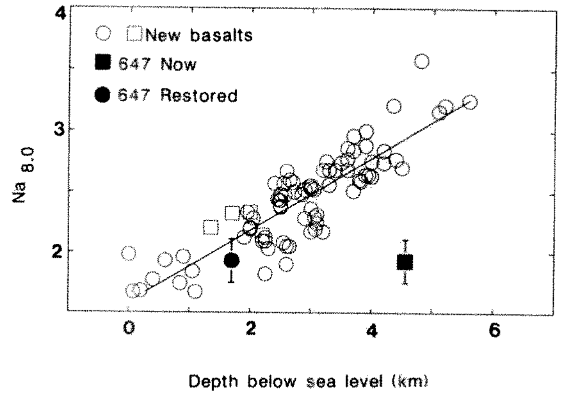

FIG. 1 The average $Na_{8.0}$ value of the 56-Myr-old basalts from Ocean Drilling Program site 647A (Na_2O wt% corrected for low-pressure fractionation[1]) plots in the field established by Klein and Langmuir[1] for zero-age axial basalts when restored to the initial water depth of site 647A (●), but not at the present depth below sea level of the basalt (■). ○, □ ('new'): ○, values for present-day ocean ridge basalts world-wide[1]; □, values described by Klein and Langmuir[1] as 'anomalous' from various axial hotspot centres. ■ ('647 now'): average value of $Na_{8.0}$ for fresh basalts from site 647A plotted at the present depth below sea level of the basalts. ● ('647 restored'): the same value plotted at the depth of the basalts restored to the initial depth at the time of formation by correcting for sediment loading and thermal subsidence (see text). Bars: one standard deviation about the mean $Na_{8.0}$ value. Lines: linear regression on the open circles plotted solely as an aid to the eye.

At their present depth below sea level, the $Na_{8.0}$ value for basalts from site 647A does not plot in the field defined by Klein and Langmuir[1]. But the value does plot in this field after the site is restored to its initial water depth by correcting for the effects of loading and thermal subsidence (Fig. 1).

The chemistry of older basalts from the other sites that we studied in the Atlantic and Indian Oceans shows the same relationship to the initial depth as it does at site 647A (Table 1; Figs 2 and 3). Figure 3 shows that values of $Na_{8.0}$, CaO/Al_2O_3 and $Fe_{8.0}$ in general fall within the fields defined by Klein and Langmuir[1] when the basalts are restored to their original water depths at the time of crustal formation. Furthermore, Fig. 3 shows that the relationship between chemistry and initial water depth at these sites holds over at least a substantial part of the range delineated by Klein and Langmuir, including shallow sites close to known hotspots such as Iceland[1].

Although the data for older basalts restored to initial water depths in general fall within the fields defined by Klein and Langmuir[1], there is only a weak inverse correlation between restored depth and $Fe_{8.0}$. This is consistent with the weaker correlation for zero-age basalts between regional averages of depth and $Fe_{8.0}$ compared with the correlations between depth and $Na_{8.0}$ or CaO/Al_2O_3 (refs 12 and 13). Klein and Langmuir[12] suggested that because iron abundances primarily reflect the pressure of melting, the weaker correlation for $Fe_{8.0}$ may reflect in part the sampling of instantaneous melts from different pressures within a zone of melting. They proposed an algorithm to isolate the component of iron variation that reflects regional differences in solidus pressure and noted a good inverse correlation between this parameter ($Fe^{G}_{8.0}$) and depth for individual analyses (Fig. 3g, h). The data for individual analyses for older basalts also show a good correlation between $Fe^{G}_{8.0}$ and restored depth, and fall within the field of data presented by Klein and Langmuir[12].

In an approach such as this, uncertainty may arise from several sources. First, the chemical composition of ocean basalts is often altered by interactions with sea water. We therefore used analyses of fresh glasses where we could, from the Smithsonian Volcanic Glass Data Base[14] and the Deep Sea Drilling Project (see refs in Table 1). These have been supplemented by whole-rock analyses of fresh rocks for the three sites representing extremes in depth where glass analyses were not available (Table 1); these bulk analyses, however, may not reflect liquid compositions because of the presence of phenocrysts in some samples. We have attempted to estimate chemical variability among samples from a single site using the standard deviation about the average values at sites where more than one group of samples was available (Fig. 3, Table 1).

Uncertainty also arises in restoration to initial depth because of the corrections for sediment loading, the effects of errors in crustal age on estimates of thermal subsidence, and the assumption that initial differences in elevation at ridge crests are preserved as the crust subsides. The correction for sediment loading depends on the average density of the sediment column[6], and this can be estimated from bulk measurements of density, the sediment thickness[7], or the two-way reflection time at the site[15]. Comparisons of the three methods at the sites that we used show that this uncertainty is in the range of only tens of metres. Similarly, errors in crustal age are usually sufficiently small that we can neglect the uncertainty they cause in restoring thermal subsidence.

The assumption that initial elevations are preserved is well established for 'normal' mid-ocean ridges and for aseismic ridges[6]. It provides a clear reference for the sites used here, and independent evidence supports many of the initial depths that we have predicted. For example, the crest of the Mid-Atlantic Ridge along the flow line east of the site AII is deeper than normal at present[16], and other studies indicate that sites near Iceland (such as 350, 407, 409) and sites younger than about 36 Myr near the Azores (559, 563, 564) should be shallow, as we find[17]. Probing the details of such depth anomalies will be interesting; for example, the original depths of sites now on swells will depend on the timing of their formation with respect to that of the swell itself.

Consequently, the correlations we see between restored initial eruption depths and major-element geochemistry—the right-hand panels of Fig. 3—lead us to suggest that our observations on basalts from older oceanic crust in the Atlantic Ocean (and from the one site in the Indian Ocean) support and extend the hypothesis of Klein and Langmuir[1] over a wide range of initial depths and age. The hypothesis seems to have survived a rather robust test.

Klein and Langmuir[1] noted a correlation between crustal thickness and basalt chemistry, and suggested that the covariations among zero-age depth, chemistry and crustal thickness result from variations in subsolidus mantle temperatures. Efforts to relate oceanic crustal thickness to other physical parameters have only rarely been successful[18,19]. Our results indicate that

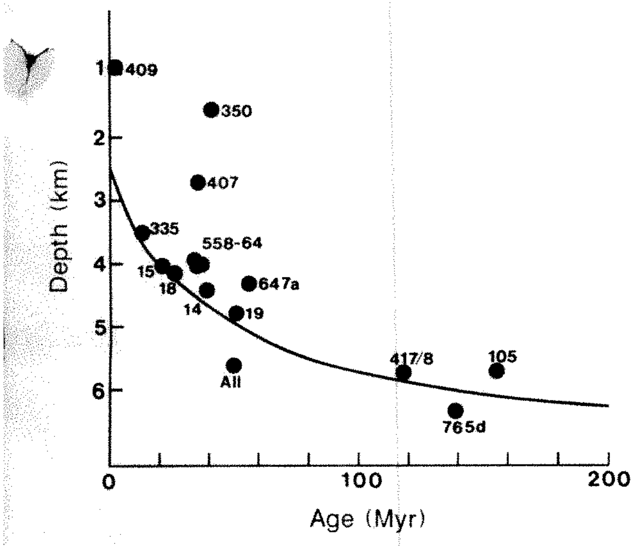

FIG. 2 The sites used in this study plotted at their unloaded depths and ages. The unloaded depth is the present depth of the basalt at a site below sea level, corrected for loading by sediments. The solid curve is the average subsidence curve for the ocean crust[8]. All locations represent Deep Sea Drilling Project or Ocean Drilling Program site numbers, except for All—a dredge haul from the western North Atlantic[34]. See Table 1 for locations.

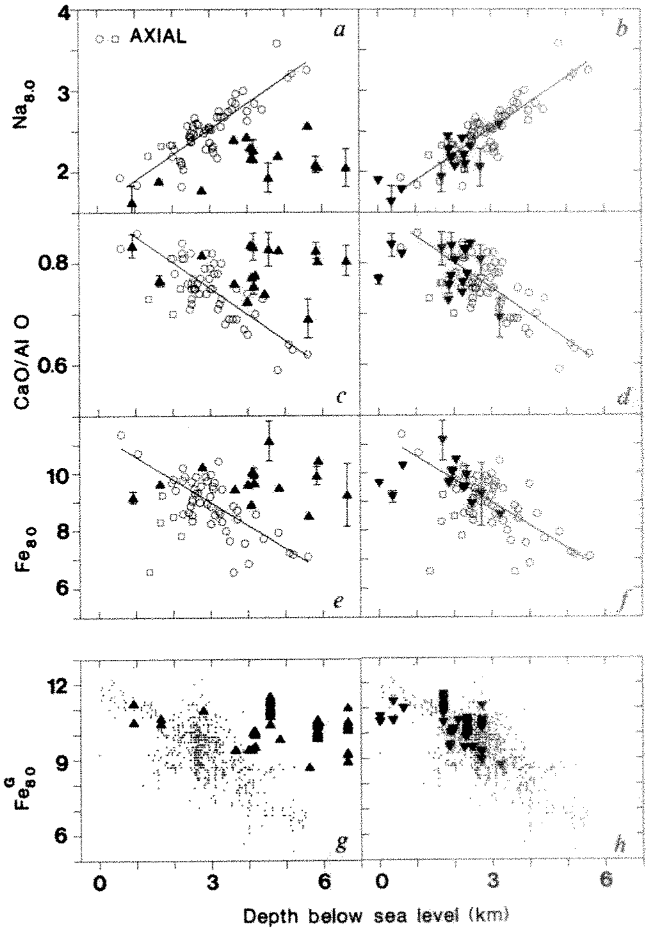

FIG. 3. The major-element geochemistry of older basalts from the mid-ocean ridges of the ocean basins correlates with the original water depth of the basalts (the depth when the basalts erupted). The variations in averaged Na_2O and FeO corrected for low-pressure fractionation ($Na_{8.0}$ and $Fe_{8.0}$), in averaged CaO/Al_2O_3, and in individual analyses of $Fe^G_{8.0}$ fall within the fields defined by Klein and Langmuir[1,12] for zero-age axial basalts after restoration to their original depths. $Fe^G_{8.0}$ is the iron variation calculated to maximize the component of iron that reflects solidus pressure[12] (see text). *a, c, e, g*, $Na_{8.0}$, CaO/Al_2O_3, $Fe_{8.0}$, $Fe^G_{8.0}$, respectively. Values from older basalts from the oceanic ridges of the Atlantic Ocean and Indian Ocean (one site) (Table 1) with standard deviations where available, plotted at their present depths below sea level (▲, 'present depth'). They do not fall into the fields defined by the modern axial basalts (○, □, dots). *b, d, f, h*, $Na_{8.0}$, CaO/Al_2O_3, $Fe_{8.0}$, $Fe^G_{8.0}$, respectively. Values from older basalts from the oceanic ridges restored to their original water depths at the time of formation by correcting present basement depths for loading by sediments and for thermal subsidence (▼, 'restored depths'). They fall within the same fields as the zero-age axial basalts. Lines: linear regression on the open circles, plotted only as an aid to the eye comparing left and right panels. *a–f*, ○ and □, ('axial'): regional averages of zero-age axial basalts[1,12] (see caption to Fig. 1). *g* and *h*, dots: individual analyses of $Fe^G_{8.0}$ for zero-age axial basalts[12].

we should extend the approach that we have devised here, and explore the relationships between crustal thickness and restored initial depth. Crustal thickness may perhaps be established more reliably on older crust than on younger crust, and so we may have opened up the possibility of more fruitful investigations of the relationships between depth, crustal thickness and crustal composition than have been possible before. Klein and Langmuir[1] also suggested that temporal variations in mantle temperatures may be explored by sampling older oceanic crust along a flow line emanating from a particular ridge segment. Our results show that the correlation between zero-age chemistry and depth can indeed be extended to older oceanic basalts, and make it attractive to seek systematic temporal variations in mantle temperature from studies of older crust.

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1. Klein, E. M. & Langmuir, C. H. *J. geophys. Res.* **92,** 8089–8115 (1987).
2. Keen, M. J. *Trans. Am. geophys. Un.* **70,** 1400 (1989).
3. Srivastava, S. P. *et al. Proc. Ocean Drilling Program, Init. Rep.* **105,** 675–725 (1987).
4. Srivastava, S. P. & Arthur, M. A. *Proc. Ocean Drilling Program, Scientific Results* **105,** 989–1089 (1989).
5. Clarke, D. B., Cameron, B. I., Muecke, G. K. & Bates, J. L. *Can. J. Earth Sci.* **26,** 956–968 (1989).
6. Sclater, J. G., Meinke, L., Bennett, A. & Murphy, C. *Mem. geol. Soc. Am.* **163,** 1–19 (1985).
7. Le Douaran, S. & Parsons, B. *J. geophys. Res.* **87,** 4715–4722 (1982).
8. Parsons, B. & Sclater, J. G. *J. geophys. Res.* **82,** 803–827 (1977).
9. Hyndman, R. D. *Can. J. Earth Sci.* **10,** 637–644 (1973).
10. Keen, C. E. & Barrett, D. L. *Geophys. J. R. astr. Soc.* **30,** 253–271 (1972).
11. White, R. & McKenzie, D. *J. geophys. Res.* **94,** 7685–7730 (1989).
12. Klein, E. M. & Langmuir, C. H. *J. geophys. Res.* **94,** 4241–4252 (1989).
13. Brodholt, J. P. & Batiza, R. *J. geophys. Res.* **94,** 4231–4239 (1989).
14. Melson, W. G. & O'Hearn, T. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 117–136 (Geol. Soc. Am., Boulder, 1986).
15. Crough, S. T. *J. geophys. Res.* **88,** 6449–6454 (1983).
16. Sclater, J. G., Lawver, L. A. & Parsons, B. *J. geophys. Res.* **80,** 1031–1052 (1975).
17. Sclater, J. G., Hellinger, S. & Tapscott, C. *J. Geol.* **85,** 509–552 (1977).
18. Purdy, G. M. & Ewing, J. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 313–330 (Geol. Soc. Am., Boulder, 1986).
19. Reid, I. & Jackson, H. R. *Mar. geophys. Res.* **5,** 165–172 (1981).
20. Maxwell, A. E. *et al. Init. Rep. DSDP* Leg 3 (1970).
21. Hollister, C. D. *et al. Init. Rep. DSDP* Leg 11 (1972).
22. Vogt, P. R. & Tucholke, B. E. (eds) *The Geology of North America, Volume M, The Western North Atlantic Region* (Geol. Soc. Am., Boulder, 1986).
23. Sclater, J. G. & Wixon, L. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 257–270 (Geol. Soc. Am., Boulder, 1986).
24. Bryan, W. B. & Frey, F. A. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 271–296 (Geol. Soc. Am., Boulder, 1986).
25. Aumento, F. *et al. Init. Rep. DSDP* Leg 37 (1977).
26. Howe, R. C. *Init. Rep. DSDP* Leg 37, 909–916 (1977).
27. Miles, G. A. *Init. Rep. DSDP* Leg 37, 929–962 (1977).
28. Talwani, M., Udintsev, G. *et al. Init. Rep. DSDP* Leg 38 (1976).
29. Luyendyk, B. P. *et al. Init. Rep. DSDP* Leg 49 (1978).
30. Donnelly, T. *Init. Rep. DSDP* Legs 51–53 (1979).
31. Bougault, H. *et al. Init. Rep. DSDP* Leg 82 (1985).
32. Ludden, J. *et al. Proc. Ocean Drilling Program Init. Rep.* **123,** 230–289 (1990).
33. *JOIDES J.* **15**(1), 3–7 (1989).
34. Ludden, J. N. & Thompson, G. *Earth planet. Sci. Lett.* **43,** 85–92 (1979).
35. Klitgord, K. & Schouten, H. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 351–378 (Geol. Soc. Am., Boulder, 1986).
36. Kent, D. V. & Gradstein, F. M. in *The Geology of North America, Volume M, The Western North Atlantic Region* (eds Vogt, P. R. & Tucholke, B. E.) 45–50 (Geol. Soc. Am., Boulder, 1986).
37. Wood, D. A. *et al. Init. Rep. DSDP* Leg 49, 597–655 (1978).

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Seismic quiescence at Parkfield: an independent indication of an imminent earthquake

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THE Parkfield segment of the San Andreas fault midway between San Francisco and Los Angeles has experienced six moderate-sized earthquakes since 1850, with an average recurrence time of about 22 years. Based on this regular behaviour, and the occurrence of the most recent earthquake in 1966, a shock of local magnitude $M_L = 5.7$ is expected to occur by 1993[1,2]. Here we suggest that a current lack of small earthquakes on this segment could be a precursory anomaly to the next characteristic Parkfield earthquake. Only four earthquakes of $M \geq 2.5$ have occurred on the segment since January 1986, whereas twenty would have been expected, extrapolating from the mean background rate. Comparison with other cases of precursory quiescence suggests that the $M = 5.7$ earthquake should occur in the next two years, rupturing the same 35-km fault segment that ruptured in 1966[3].

Seismic quiescence is a phenomenon that has been used successfully to predict moderate and large earthquakes[4-6]. Seismicity consists of two parts: events that depend on each other (clusters) and independent events. After removing the clusters from the data[7], the remaining seismicity (background) shows a remarkably constant rate over many years. Against this constant rate a decrease by about a factor of two (seismic quiescence) that lasts for approximately a year or more is an unusual occurrence and is often followed by a mainshock[8]. The quiescence is thought to signal a change in the physical process governing the production of small earthquakes in the rupture volume. Usually the quiescence outlines the mainshock rupture volume and affects all magnitude bands[4-6,8-11] but it has been proposed that in some cases the rate of small-magnitude earthquakes remains constant[12,13].

An example of precursory seismic quiescence is shown in Fig. 1*a*. The Stone Canyon case was used as a basis for estimating what to expect in the Parkfield area because it occurred on the San Andreas fault about 80 km northwest of Parkfield and comes from a similar tectonic environment.

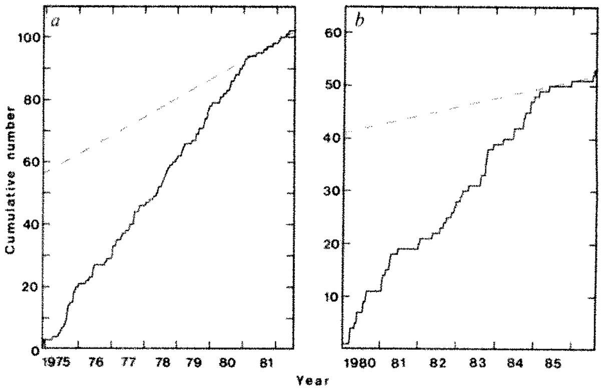

FIG. 1 Applicability of the quiescence hypothesis to the Parkfield segment of the San Andreas fault, tested by comparing the patterns of seismicity rate in the source volumes of mainshocks at *a*, Stone Canyon and *b*, Parkfield. The mainshocks ($M = 5$ at Stone Canyon, $M = 3.6$ at Parkfield) occurred at the time of the right end of the plots of cumulative seismicity (all shocks $M > 1.8$ at Stone Canyon and $M > 0.9$ at Parkfield) and quiescence started 1.5 years before the mainshocks. The slope of these curves is the mean seismicity rate. The dashed lines extrapolate the anomalously low precursory rate back in time for comparison with the observed background rate. The similarity of the pattern strongly suggests that the quiescence hypothesis should be as applicable at Parkfield as at Stone Canyon, where a successful prediction was made[4].

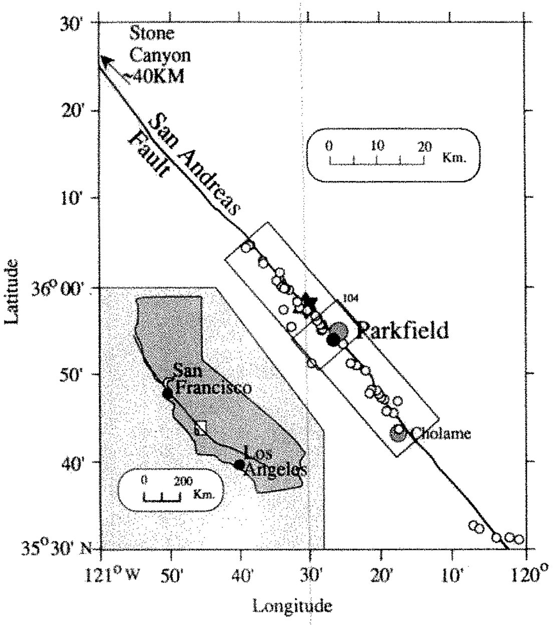

FIG. 2 Map of the Parkfield segment of the San Andreas fault showing the epicentres of earthquakes with $M > 2.4$ that occurred between 1975 and 1985. Since the beginning of 1986 only four such earthquakes occurred. The star and solid dot denote the mainshock epicentres of 28 June 1966 and 29 August 1986 respectively. The large rectangle outlines the area for which the cumulative seismicity curves are shown in Fig. 3, the small rectangle (104) outlines the area used for the earthquake count in Fig. 1*b*. The quiescence extends along the fault from latitude 35.7° N to 36.05° N approximately but is most pronounced north of Parkfield.

The macroseismic observations and surface-wave records seem to indicate that the Parkfield fault segment ruptures repeatedly in earthquakes with practically the same location, fault length and fault slip (a characteristic earthquake)[1]. Based on the periodicity with which the last five such earthquakes have occurred[1], the next characteristic Parkfield earthquake was expected to occur between 1983 and 1993. This event has not taken place yet.

Seismicity near Parkfield is monitored by the US Geological Survey, which publishes an earthquake catalogue for the area outlined in Fig. 2. Artificially introduced rate changes through inadvertant changes of the magnitude scale or of the detection capability, which are present in all the catalogues we have examined, can sometimes be identified using the magnitude signature method[9,10,14–18]. The details of our estimate of the minimum magnitude of homogeneous recording as a function of time, and the artificial magnitude shifts in the Parkfield catalogue will be given elsewhere.

The cumulative number of earthquakes for a subsegment of the fault is shown in Fig. 1*b*. The segment is 10 km long and randomly placed, covering part of the rupture of one of the two largest earthquakes that took place in the past ten years in the Parkfield area (29 August 1986, $M = 3.6$). The figure shows that quiescence preceded this event, and a more detailed analysis shows that it could have been predicted generating only one false alarm, using an alarm level from the Stone Canyon case history (Fig. 1*a*). We therefore conclude that the quiescence hypothesis is applicable to the Parkfield area[19].

The present seismicity rates for earthquakes with $M > 2.4$ and $M > 2.0$ are compared to the background rate in Fig. 3. It is obvious that the rate has decreased by 80 and 46%, respectively. This quiescence is present in all of the Parkfield segment (Fig. 2) except in the northernmost few kilometres. The onset of the quiescence is September 1986 for $M > 2.0$ events (for $M > 2.4$ it is January 1986) so that, at the time of writing, it has lasted ~3.5–4 years.

How should this quiescence anomaly be interpreted? The magnitude signature indicates that it is not artificial and the workers producing the catalogue, who also noticed the quiescence[20], do not think that the observed rate decrease in events of relatively large magnitude can be artificial. We conclude that the current Parkfield quiescence is probably natural and may be interpreted using the quiescence hypothesis. Based on the criteria from the Stone Canyon case we judge the current Parkfield quiescence significant. In the north the anomaly seems to be terminated near latitude 36.05° N. The southern extent of the anomaly cannot be well defined because the number of earthquakes per volume decreases towards the south to near zero at the southern end of the polygon shown in Fig. 2.

All aspects of the observed anomaly agree with the quiescence hypothesis, except that it is observed only for earthquakes of $M > 2$ and not for smaller events. The long duration of the anomaly, and its large spatial extent (~40 km along the fault) strongly suggest that this anomaly is part of the preparatory process for a fairly large rupture. We therefore conclude that the observed quiescence heralds the expected Parkfield earthquake ($M_L = 5.7$), not a smaller mainshock.

The time window for the expected earthquake was ten years[1,2], almost 50% of the recurrence period. We believe we can reduce this. In a previous test of the quiescence hypothesis in real time, we had predicted that two moderate earthquakes would rupture the San Andreas fault along specific segments[21]. One of these predictions was incorrect, the other was fulfilled by a rupture of the exact fault segment specified[4]. In that case the anomalous fault segment was a factor of four shorter than at Parkfield. At the time when the prediction was made the anomaly had lasted 1.9 years and the uncertainty of the duration was estimated to be ±0.5 yr (ref. 21).

In the Aleutian islands a large earthquake was predicted based on quiescence[5,6] that had lasted 3.5 years and which had been terminated by an $M_s = 8$ mainshock (M_s is the surface wave magnitude). In Hawaii $M_s = 6.6$ and $M_s = 7.2$ earthquakes were preceded by precursory quiescences of 2.4 and 3.8 years[10,11]. Comparing the present quiescence of 3.5 years at Parkfield with these values indicates that the Parkfield earthquake must be

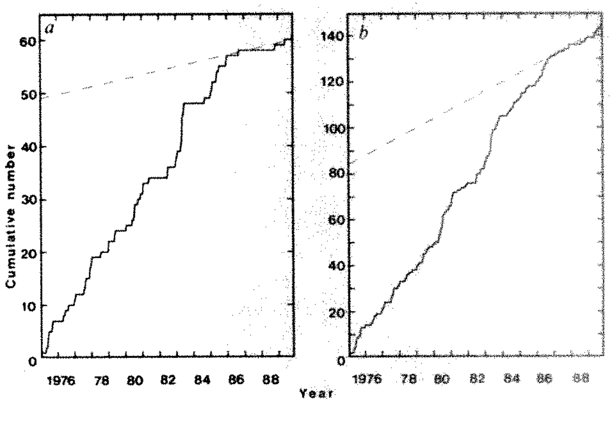

FIG. 3 Cumulative number of earthquakes as a function of time for the Parkfield area outlined by a rectangle in Fig. 2 for *a*, $M > 2.4$ and *b*, $M > 2.0$. The current, anomalously low seismicity rate is extrapolated back in time by a dashed line for comparison with the much larger observed background rate, represented by the slope of the solid curve. Because this pattern is very similar to that observed before other mainshocks along the San Andreas fault (Fig. 1) we interpret it to indicate that the next Parkfield earthquake will occur within the next 2 years. The rates decreased by 80% and 40% respectively for $M > 2.4$ and $M > 2.0$ earthquakes.

imminent. Along the San Andreas fault (Stone Canyon and Parkfield), however, quiescences of 1.5, 2.9 and 1.5 years were observed before mainshocks with magnitudes of 5.0, 4.7 and 3.6, respectively. It seems that along this segment of the San Andreas fault quiesences last longer than at other locations for mainshocks of the same magnitude.

Many workers have suggested that the duration of precursory quiescence should increase with increasing magnitude. The data suggest that this may be so, but are not conclusive[8]. In Hawaii an increase of one order in magnitude increases the duration of quiescence by about 1.5 years. The next characteristic Parkfield earthquake is expected[1,2] to have a magnitude about two units larger than the prototype event in Parkfield (Fig. 1*b*, $M_{\mathrm{L}} = 3.6$). Thus one may propose that the quiescence before the next characteristic earthquake should be about 3 years longer than the quiescence before the $M_{\mathrm{L}} = 3.6$ event. This would lead to an expected precursor duration for the Parkfield earthquake of ~4.5 years. It is not possible to calculate rigorously an error for the expected precursor time because few data points are available. From the variations of precursor times for earthquakes along the San Andreas fault and the differences in their magnitudes, we estimate that the uncertainty in the expected precursor time may be about one year.

We feel that the above inferences are weak, but this is unavoidable in the absence of more data. We therefore estimate the occurrence time of the next Parkfield earthquake as March 1991 ±1 year (4.5 years after September 1986, the average onset time of the anomaly). The magnitude and location of this event were given by Bakun and McEvilly[1].

The estimate of the probability with which the characteristic Parkfield earthquake is expected within the next 2 years (our range), without the information presented here, depends on the assumptions made. If the six known Parkfield mainshocks since 1850 were all characteristic events and are assumed to occur at random times, there is an 8% chance for the next one in any 2-year period. But if one uses the method of Nishenko and Buland[22] or of Davis *et al.*[23] to analyse the recurrence time, the estimate is 38 and 14%, respectively. Based on our systematic searches for quiescent periods that do not precede mainshocks, we estimate that there is a less than 50% chance that the quiescence at Parkfield is a false alarm. Thus, the probability gain owing to seismic quiescence ranges from a factor of 1.3 to 3, depending on the assumptions.

The time window of uncertainty proposed by us is two years, which is one-fifth of the original ten-year window but, because only four years of the original window are left, this time is only shortened by 50%. Thus the information contained in the decrease of the seismicity rate is useful, even though the quiescence method is in its infancy. The fact that our time window falls within the original window is encouraging. As the two methods of estimating the occurrence time of the next Parkfield earthquake are based on independent data and independent models, the agreement in their results represents strong mutual support, and increased confidence that the Parkfield earthquake will occur soon. Other supporting evidence comes from a decrease in shortening rate for geodetic lines crossing the fault north of Parkfield in mid-1986. This observation, which will be reported in detail elsewhere[24], also indicates that a change in the faulting process took place in mid-1986. We interpret these two phenomena as intermediate-term precursors. Other intermediate-term precursors, if they exist in other data sets should also show up in mid-1986, and short-term precursors may still occur in the future, allowing a further reduction in the time window of the prediction of the next Parkfield earthquake. □

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1. Bakun, W. H. & McEvilly, T. V. *J. geophys. Res.* **89**, 3051–3058 (1984).
2. Bakun, W. H. & Lindh, A. G. *Science* **229**, 619–624 (1985).
3. Brown, R. D. Jr *et al. Geol. Surv., prof. Pap. U.S.* **597** (1967).
4. Wyss, M. & Burford, R. O. *Nature* **329**, 323–325 (1987).
5. Kisslinger, C., McDonald, C. & Bowman, J. R. *Proc. IASPEI* 32 (1985).
6. Kisslinger, C. *Bull. seism. Soc. Am.* **78**, 218–229 (1988).
7. Reasenberg, P. *J. geophys. Res.* **90**, 5479–5495 (1985).
8. Wyss, M. & Habermann, R. E. *Pure appl. Geophys.* **126**, 319–332 (1988).
9. Wyss, M. & Habermann, R. E. *Pure appl. Geophys.* **126**, 333–356 (1988).
10. Wyss, M. *Bull. seism. Soc. Am.* **76**, 785–800 (1986).
11. Wyss, M., Klein, F. W. & Johnston, A. C. *J. geophy. Res.* **86**, 3881–3900 (1981).
12. Cao, T. & Aki, K. *Geol. Surv. U.S., Open file Rep. 87-591* 934–957 (1987).
13. Snoke, J. A., Taylor, D. W. A. & Sacks, I. S. *Eos* **70**, 1229 (1989).
14. Habermann, R. E. *J. geophys. Res.* **88**, 5056–5064 (1983).
15. Habermann, R. E. *Pure appl. Geophys.* **126**, 279–318 (1988).
16. Habermann, R. E. *Bull. seism. Soc. Am.* **72**, 93–112 (1982).
17. Habermann, R. E. *Bull. seism. Soc. Am.* **76**, 1660–1667 (1986).
18. Habermann, R. E. & Wyss, M. *Bull. seism. Soc. Am.* **74**, 1743–1755 (1984).
19. Bodin, P., Wyss, M. & Habermann, R. E. *Eos* **70**, 1230 (1989).
20. Aviles, C. A. & Valdes, C. M. *Eos* **70**, 1228 (1989).
21. Wyss, M. & Burford, R. O. *Geol. Surv. U.S. Open File Rep. 85-745* 367–426 (1985).
22. Nishenko, S. P. & Buland, R. *Bull. seism. Soc. Am.* **77**, 1382–1399 (1987).
23. Davis, P. M., Jackson, D. D. & Kagan, Y. Y. *Bull. seism. Soc. Am.* **79**, 1439–1450 (1989).
24. Wyss, M., Slater, L. & Burford, R. O. *Nature* **345**, 428–431 (1990).

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Decrease in deformation rate as a possible precursor to the next Parkfield earthquake

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CRUSTAL deformations along fault zones are monitored because they show the accumulation and possible premonitory release of strain energy before major earthquakes. On the Parkfield segment of the San Andreas fault the next mainshock (local magnitude $M_{\mathrm{L}} = 5.7$) is expected to occur between 1983 and 1993, based on the relatively regular recurrence of characteristic earthquakes in 1857, 1881, 1901, 1922, 1934 and 1966[1]. A concerted monitoring effort is therefore underway at Parkfield, with the aim of detecting precursory changes in observable quantities that can be measured reliably[2]. Here we report data from two geodetic lines crossing the San Andreas fault near Parkfield which show that the rate of shortening decreased by ~20% starting in August 1986. This change coincided with a rate decrease of 45% for earthquakes of $M_{\mathrm{L}} \geqslant 2.1$ in the area[3,4]. Both phenomena may have been caused by temporary locking of the fault surface, or a decrease in strain accumulation rate, and they are interpreted as intermediate-term precursors to the next Parkfield earthquake.

The monitoring of crustal deformations near the fault using a two-colour laser distance measuring instrument was begun in 1984. The accuracy of the measurements is 10^{-7} times the line length[5,6] thus the repeatability for a 5-km-long line is 0.5 mm. The instrument, located near the centre of the 1966 rupture zone (Fig. 2), simultaneously transmits two laser beams (wavelengths 441.6 nm and 632.8 nm) to reflectors that return them to the observation site, where the two-way travel times are measured. The slight difference in travel times between the two beams allows highly accurate corrections for changing atmospheric conditions[5].

The lengths of the lines have been measured on about 700 days since mid-1984 (almost three times per week). Seasonal fluctuations, observed for most line lengths[7], can be as large as 5 mm for the line most strongly affected. For least-squares straight-line fits to the data from lines not strongly affected by seasonal changes, the standard deviations range from 0.06 to 0.2 mm yr^{-1}. We should therefore be able to resolve changes in deformation rate of less than 1 mm yr^{-1}.

The length changes for lines crossing the San Andreas fault (Fig. 2) were examined carefully (Fig. 1*a*) after it was discovered that seismic quiescence had started in the Parkfield area in September 1986[3,4,8]. This attempt to identify a geodetic precursor was motivated by the successful prediction of an earthquake ($M_L = 4.7$, May 1986) on the San Andreas fault 80 km northwest of Parkfield, based on simultaneous precursory seismic quiescence and reduction in fault-creep (aseismic slip along the fault surface)[9,10].

To test the hypothesis that the deformation rate near Parkfield may have been reduced synchronously with the seismic quiescence, we estimated the average shortening rate for early and late sections of the record by a least-squares linear fit of both. The comparison was made for divisions between the two sections at every measurement in Fig. 1*a*, starting with the eightieth point and ending with the eightieth-before-final data point. The division points that yielded the largest contrast between early and late sections were in August 1986 for all four data sets of Fig. 3. Thus the shortening rate decrease and the seismic quiescence started at the same time within the resolving power of the data, which is estimated to be no better than one month and several months for the seismicity and deformation rates, respectively. The decreases in rate are 1.72 and 1.67 mm yr^{-1} for CAN and BAR respectively, and the standard deviations for the straight-line fits before and after August 1986 are 0.15 and 0.05 mm yr^{-1} respectively for both lines. If we assume that displacements are parallel to the fault, the average decrease in deformation rate is estimated to be ~2.1 mm yr^{-1}.

The difference in shortening rate before and after August 1986 at BAR and CAN (10.1 to 8.4 mm yr^{-1}) is significant because it is more than 10 times the standard deviations of the slope changes between the two segments of the data. We therefore feel that the data present a strong case for a real change in the relative displacement rates of BAR and CAN.

To reduce or eliminate possible errors (mistakes in meteorological measurements, instrument instability or electronic drift) we calculated the difference between the distances of two lines extending in similar directions but to benchmarks on opposite sides of the fault, measured at nearly the same time (within minutes). The differences BAR − LANG and CAN − LANG (Figs 1 and 3 respectively) show the rate change even more clearly. If we assume that changes in the difference between these lines is the result of relative displacement of the two benchmarks BAR and LANG parallel to the fault, we calculate that the differential displacement rate changed from 16.3 to 13.0 mm yr^{-1} in August 1986. The interruption of the time series

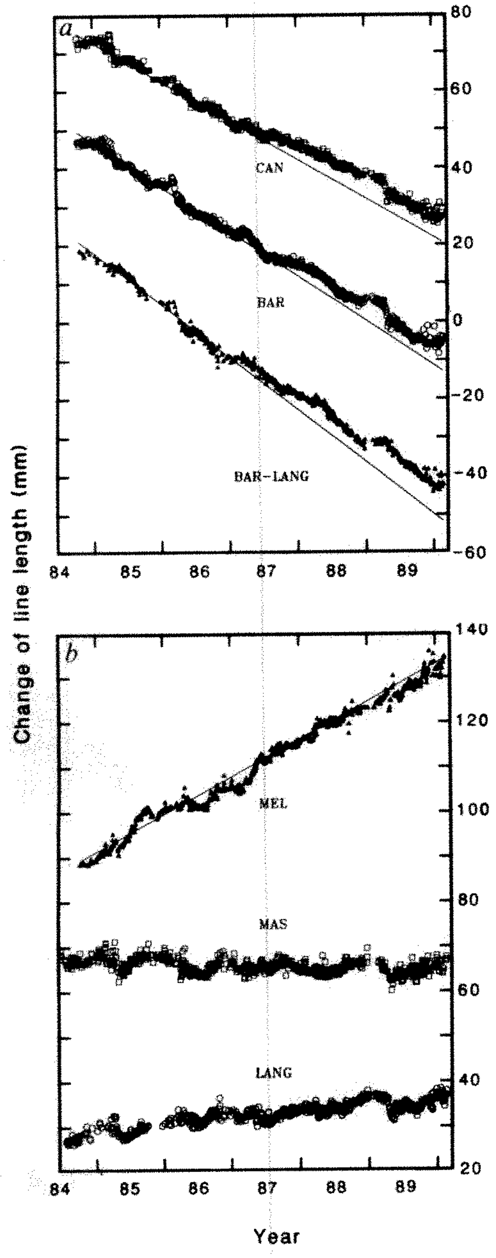

FIG. 1 *a*, Change of length as a function of time for two lines crossing the San Andreas observed from CAR to benchmarks situated north of Parkfield. Squares and circles are for lines to CAN and BAR respectively (Fig. 2). Straight lines show the least-squares fits for the period between the start of the data and August 1986. The shortening of these lines indicates fault slip or strain accumulation with the eastern side of the fault moving to the southeast relative to the observation site at Car Hill. In the differences between lengths to BAR and LANG (triangles), errors caused by meteorological influence, instrument movement or electronic instabilities are removed to a great extent. *b*, Change of length for two lines that do not cross the fault (LANG and MAS) and for one line that crosses the fault to the south (MEL).

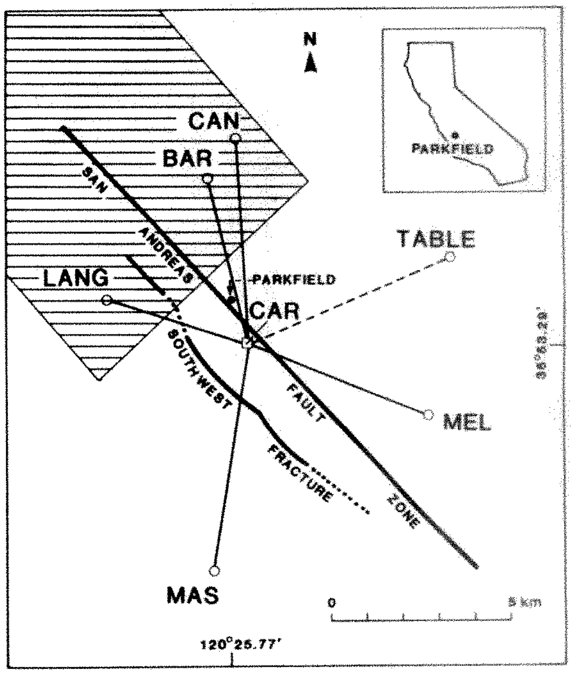

FIG. 2 Map of the Parkfield area with solid lines connecting the two-colour laser observation site CAR with the reflector points for which line-length data are shown in Fig. 1. The approximate location of the San Andreas fault is also indicated. The fault segment with the most pronounced seismic quiescence is hatched. The length of the dashed line was affected by the Kettleman Hills earthquake of 1985[7].

for the baseline to CAN[7] does not seem to be a source of error because the data for CAN and BAR give the same results.

The change of displacement rate is best seen in plots of the residuals with respect to the straight-line fits through August 1986 (Fig. 3). At present, a shortening deficiency of ~5 mm has accumulated (7 mm projected as displacement deficiency parallel to the fault). The accumulated differential displacement deficiency (BAR − LANG and CAN − LANG) is ~10 mm. We conclude from this data analysis that in the later part of 1986 a decrease in the displacement rate of ~20% took place on the San Andreas fault northwest of Parkfield.

We could not detect significant changes in rates of shortening or lengthening in other lines during 1986 and 1987. Examples of length changes for lines not crossing the fault to bench marks with small seasonal changes are shown in Fig. 1*b*. The fault-crossing line south of the observation station (CAR to MEL in Fig. 2) did not show a significant difference in the fits for the early and late sections. Thus we propose that the deformation rate change took place only northwest of Parkfield. This suggestion is also in agreement with the observations of the seismicity rate, which indicate that the seismic quiescence was most strongly developed in the fault segment northwest of Parkfield[3,4] as indicated by the hatched rectangle in Fig. 2. The data for other lines[7] are not discussed here because they either start too late or contain strong seasonal changes.

No moderate to large earthquakes took place in the vicinity of Parkfield within a year before and since the time when the observed decrease in displacement rate took place. Before the two-colour laser was installed, the Coalinga earthquake (M_L = 6.9, 2 May 1983, located ~45 km from Parkfield off the San Andreas fault) caused a perturbation in the creep observed along the Parkfield segment of the fault[11,13]. The Kettleman Hills earthquake ($M_L = 5.5$, 4 August 1985, located ~35 km to the northeast of Parkfield) changed the laser line length to TABLE, but otherwise had only small effects on line lengths and creep records[12]. Therefore we know that tectonic events at some distance can influence the slip rate along the Parkfield fault segment, but we see no external cause to which the August 1986 lasting rate decrease could be attributed. Also it is not likely that changing moisture content of the ground could have caused the lasting rate change because the average annual amount of rainfall during the laser observation period was the same, within 10%, before and after August 1986.

Aseismic slip measured at the surface by creepmeters does not show a decrease in rate at any time between 1984 and 1989 but there may have been an acceleration in early 1986 at a creepmeter south of Parkfield[7]. Langbein *et al.*[7] modelled the observed deformations in the laser network by varying fault slip at depth as a function of space and time. Their Fig. 10 shows that, according to their model, a decrease of fault slip rate occurred north of Parkfield in 1986.

Partial locking of the fault[13] could decrease both the rate of seismic and aseismic slip. But this mechanism would lead to an increase in ambient stress which should result in a decrease in *b*-value[14] (equivalent to an increase in average magnitude of earthquakes produced), yet the opposite is observed: the *b*-value increased from 1.1 to 1.4 as we shall show elsewhere. If an increase in *b*-value is taken to signal a decrease in ambient shear stress, or an increase in pore pressure[15], it seems difficult to explain the reduction in deformation rate, because the latter should increase if pore pressure is increased or if strain is released. The mechanism that causes seismic quiescence and deformation rate decrease cannot be ascertained yet because not enough constraints exist.

The seismic quiescence and the reduction in fault slip now in effect at Parkfield show patterns very similar to those observed before the successfully predicted Stone Canyon earthquake[9,10]. We propose that these two phenomena strongly suggest that an intermediate-term preparatory process to a characteristic Parkfield earthquake[1] started in late 1986, and that this earthquake is to be expected soon. We have argued that, based on comparisons with other case histories of seismic quiescence, the Parkfield anomaly has already lasted for an unusually long period, therefore the mainshock is expected in the near future, and no later than March 1992 (ref. 4).

The occurrence of deformation rate decrease as an intermediate-term precursor does not preclude accelerated slip during weeks to days before the next mainshock as expected on the basis of some models of the failure process[16]. The possibility of a short-term increase in slip rate is suggested by the observa-

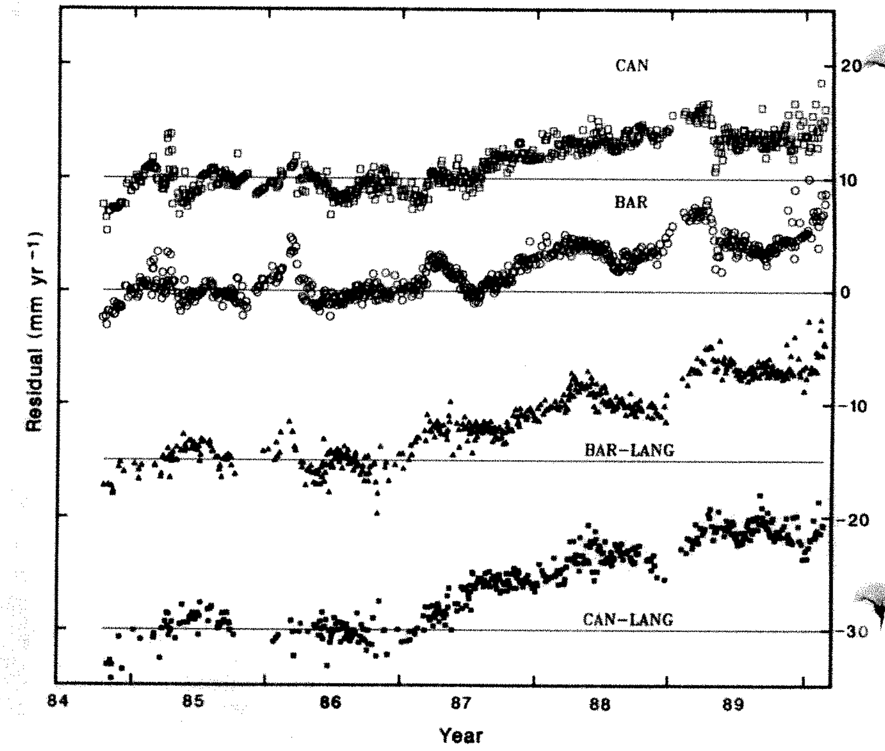

FIG. 3 Residuals of the rate of length changes of the lines to CAN (squares), BAR (circles) and the difference BAR − LANG (triangles), CAN − LANG (squares) with respect to the least-squares fits of data for the period between October 1984 and August 1986. Changes of line length are shown in Fig. 1 and locations of the lines are given in Fig. 2.

tion of relatively fresh en echelon cracks along the fault during the month before the last Parkfield mainshock[17], and by the rupture of a water main only hours before it[18]. The relationship between intermediate-term precursors (months to years) and short-term precursors (hours to weeks) is unknown.

It is important that independent precursors are observed in order to gain confidence during the process of refining an earthquake prediction. The observations of seismic quiescence and deformation deficiency are independent and are affected by different sources of error. Thus their nearly synchronous onset strongly suggests that these lasting changes are real. The two types of changes are probably not physically independent, however, because a common underlying process may cause both phenomena.

It is also important to attempt the identification of precursors before the mainshock has occurred in order to learn what misinterpretations are made when one is faced with data which may contain potential precursors. This attempted earthquake prediction is notable in being based on four lines of evidence: regular recurrence, seismicity rate decrease of 45%, deformation rate decrease of 20% and a *b*-value increase of 25%. ☐

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1. Bakun, W. H. & McEvilly, T. V. *J. geophys. Res.* **89,** 3051–3058 (1984).
2. Bakun, W. H. & Lindh, A. G. *Science* **229,** 619–624 (1985).
3. Bodin, P., Wyss, M. & Habermann, R. E. *Eos* **70,** 1230 (1989).
4. Wyss, M., Bodin, P. & Habermann, R. E. *Nature* **345,** 426–428 (1990).
5. Slater, L. E. & Huggett, G. R. *J. geophys. Res.* **81,** 6299–6306 (1976).
6. Burford, R. O. & Slater, L. E. *U.S. Geol. Survey, Open File Report, 85-754,* 117–129 (1985).
7. Langbein, L. E., Burford, R. O. & Slater, L. E. *J. geophys. Res.* **95,** 2533–2552 (1990).
8. Aviles, C. A. & Valdes, C. M. *Eos* **70,** 1228 (1989).
9. Wyss, M. & Burford, R. O. *U.S. Geol. Survey, Open File Report 85-745,* 367–426 (1985).
10. Wyss, M. & Burford, R. O. *Nature* **329,** 323–325 (1987).
11. Burford, R. O., Schulz, S. S. & Simpson, R. W. *U.S. Geol. Survey Open File Report 87-591,* 845–867 (1987).
12. Simpson, R. W., Schulz, S. S., Dietz, L. D. & Burford, R. O. *Pure appl. Geophys.* **126,** 665–685 (1988).
13. Burford, R. O. *Pure appl. Geophys.* **126,** 499–529 (1988).
14. Scholz, C. H. *Bull. seism. Soc. Am.* **58,** 399–416, (1968).
15. Wyss, M. *Geophys. Jl R. astr. Soc.* **31,** 341–359 (1973).
16. Stuart, W. D. *J. geophys. Res.* **84,** 1063–1070 (1979).
17. Allen, C. R. & Smith, S. W. *Bull. seism. Soc. Am.* **56,** 966–967 (1966).
18. Brown, R. D. Jr *et al. Geol. Surv. prof. Pap. U.S.* **597,** (1967).

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Biological recovery of an acid lake after reductions in industrial emissions of sulphur

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THERE is now considerable evidence from Canada[1–4], Norway[5], Sweden[6] and Scotland[7] that lake and stream acidification is chemically reversible. Water quality improvements have been shown to occur rapidly when inputs of sulphur are reduced[8]. However, there is little evidence that biological systems recover after emission reductions[9]. We report here on changes in the biological community of an acid-stressed lake near the metal smelters at Sudbury, Canada, during a 10-year period (1978–1988) after smelter emissions of sulphur were reduced by more than 50%. Our studies of the fish, benthic invertebrates, and zooplankton in this lake show that rapid recovery and reinvasion occurs among some acid-sensitive species.

The study was conducted on Whitepine Lake (47°17′N, 80°50′W), a clear, dilute (conductivity around 30 μmho cm^{-1}) headwater lake (surface area 67 ha, maximum depth 22 m), located in a forested area 90 km north of Sudbury. The lake receives both wet and dry inputs of sulphur, but is outside the zone of high deposition of metal particulates from the Sudbury smelters[1] (water concentrations of Cu and Ni $<2\ \mu g\ l^{-1}$). In 1980 the lake was acidic (pH$<$5.5) and its fish population showed classical signs of acid stress: acid-tolerant yellow perch (*Perca flavescens*) were very abundant, while acid-sensitive species such as lake trout (*Salvelinus namaycush*), burbot (*Lota lota*), and white suckers (*Catostomus commersoni*) were extremely rare and were not reproducing[10].

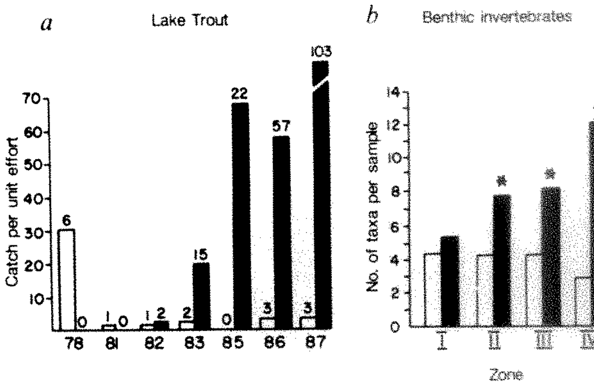

FIG. 1 *a*, Catch per unit of effort. Number of lake trout caught in experimental gillnets (1.8–7.6-cm mesh) in Whitepine Lake. Data standardized to 100 h of netting effort. Only naturally produced fish are included in the figure. The total number of adults (open columns) and juveniles (solid columns) captured in each year is indicated. *b*, Changes in the number of taxa of benthic invertebrates in Whitepine Lake. Three replicate samples were collected at each of three stations in each offshore zone (I, 8–21 m; II, 4–8 m; III, 1.5–4 m) using an 15 × 15 cm Ekman dredge on May 20, in both 1982 (open columns) and 1988 (solid columns). Samples were sieved through a 600-μm mesh screen before counting. Sampling was repeated in October of both years with similar results. The nearshore rocky areas (IV, $<$1 m) were sampled with artificial substrates (23 × 23 × 15 cm cages filled with 5–10-cm diameter clean stones) placed in triplicate at five sites. The placement and retrieval dates were 25 May and 19 July, respectively, in both 1983 (open columns) and 1988 (solid columns). Autumn samples were also collected using artificial samplers; again with similar results.
* Depth zones showing significant increases (*t*-test, $P \leq 0.05$) in number of taxa between sampling periods.

Monitoring of lake water quality began in 1980. Fish populations were assessed during 1978, 1981, 1982, 1983, 1984, 1986, and 1987 using similar sampling sites and capture gear (variable mesh gillnets, trapnets, plexiglas minnow traps)[11]. Benthic invertebrates inhabiting soft sediments were sampled in 1982 and 1988, with a depth-stratified sampling design and an Ekman dredge collector. Artificial substrate collectors were used in 1983 and 1988 to sample invertebrates that occupied rocky nearshore areas. Zooplankton were collected at least monthly during the open water periods of 1980–1988. Zooplankton samples were collected with a 12.5-cm diameter 80-μm mesh plankton net drawn vertically through the water column at a mid-lake site.

Water quality improved significantly ($P<0.01$) from 1980 (pH 5.4, acid-neutralizing capacity (ANC) 1 μequivalent l^{-1}) to 1988 (pH 5.9, ANC 11 μequivalent l^{-1}) (Table 1). Specific conductance and concentrations of SO_4, Ca and Al all declined with reductions in industrial emissions of SO_2 (Table 1).

The first evidence of biological recovery was the appearance of naturally produced young lake trout from the remnant adult population. Young lake trout first appeared in 1982 and became increasingly abundant throughout the rest of the study (Fig. 1). Production of young trout by the remnant adult population was discovered during routine monitoring of hatchery fish added to this lake in 1980 and 1981[11]. Each hatchery fish was marked before addition by removing the adipose fin. During monitoring of these individuals an increasing number of unmarked fish

TABLE 1 Water quality data for Whitepine Lake, 1980–1988

	Sudbury emissions of SO_2 ($\times 10^3$ tonnes)*	pH	Acid-neutralizing capacity (μequivalent l^{-1})	Conductivity (μmho cm^{-1})	Ca (μequivalent l^{-1})	SO_4 (μequivalent l^{-1})	Total Al ($\mu g\ l^{-1}$)
1980	935	5.4 (5.3–5.8)	1 ± 5	37 ± 2	156 ± 18	237 ± 20	69 ± 20
1981	837	5.7 (5.6–5.8)	4 ± 2	35 ± 1	145 ± 31	235 ± 12	59 ± 21
1982	389	5.8 (5.8–5.9)	10 ± 4	36 ± 3	138 ± 17	218 ± 20	44 ± 15
1983	538	5.8 (5.7–5.8)	8 ± 3	32 ± 1	123 ± 5	198 ± 22	36 ± 12
1984	767	5.8 (5.7–5.8)	8 ± 2	32 ± 2	125 ± 10	198 ± 24	40 ± 15
1985	769	5.8 (5.7–5.9)	12 ± 4	31 ± 1	119 ± 13	195 ± 4	50 ± 11
1986	725	5.9 (5.8–5.9)	12 ± 4	29 ± 1	120 ± 9	177 ± 17	34 ± 10
1987	730	6.0 (5.9–6.1)	15 ± 1	30 ± 4	115 ± 13	196 ± 23	26 ± 11
1988	718	5.9 (5.9–6.0)	11 ± 3	30 ± 4	115 ± 6	195 ± 10	40 ± 14

Annual means (±1 s.d., range given for pH) are calculated from monthly data ($n = 4$–11) for samples collected through the epilimnion and metalimnion. Changes through time were determined to be significant ($P < 0.01$) for all of the above chemical parameters using linear correlation analysis of log-transformed (except pH) data against time in months.

* Annual averages for SO_2 emissions ($\times 10^3$ tonnes) before the study were: 1960–64, 2,241 tonnes; 1965–69, 2,202 tonnes; 1970–74, 1,867 tonnes; 1975–79, 1,064 tonnes.

occurred in yearly catches. By 1987, the naturally produced fish were far more abundant than the stocked fish, and dominated (87%) the gillnet catches. These young fish began to appear at least two years before the stocked fish had reached sexual maturity (age 5, Autumn 1984).

Other researchers have shown that lake trout do not reproduce when pH drops below 5.5–5.6 (refs 12–13). Our study showed that this effect is reversible; successful reproduction occurred when pH increased above 5.5.

Successful reproduction did not occur in the white sucker and burbot populations, and these species were not observed after 1984. Very few adults (less than five fish observed) were present for each species, a fact that presumably contributed to their extinction. In a lake where adult white suckers were abundant and were not reproducing under low pH conditions, successful reproduction has been shown to occur with an increase in pH (ref. 14).

The rapid increase in abundance of lake trout, an omnivorous predator, caused marked changes in prey populations. Trapnet catches of perch, a common prey species of lake trout[15], declined from 145 per night in 1982 to approximately 2 per night in 1987. Densities of benthic invertebrates in the deep zones of the lake (8–21 m) that are occupied by lake trout declined from 1140 m^{-2} in 1982 to 650 m^{-2} in 1988. In contrast, the density of invertebrates in the shallow areas (0–8 m), the principal foraging area of perch, increased with the decline in abundance of perch. Soft sediment areas provided 550 invertebrates m^{-2} in 1982, 1,320 m^{-2} in 1988; rocky areas, 6 invertebrates per sample in 1983, 164 per sample in 1988.

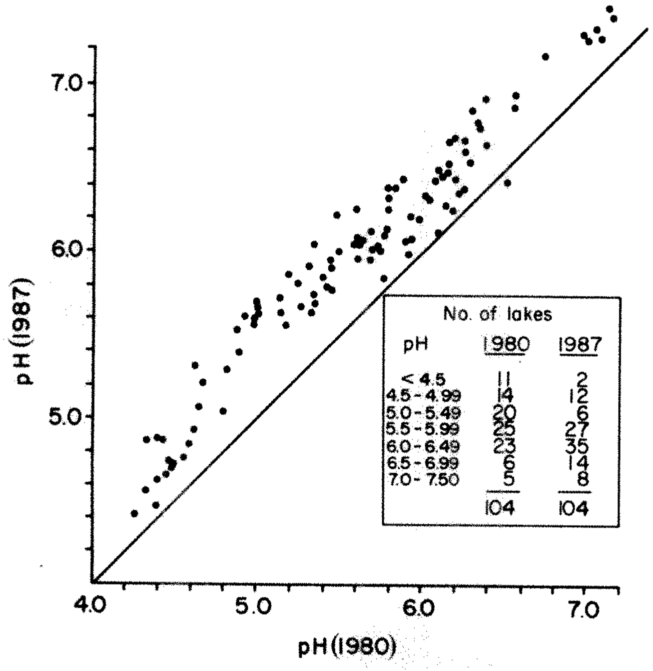

FIG. 2 Change in pH of 104 lake-trout lakes near Sudbury, Canada between 1980 and 1987. Water samples were collected with a 5-m tube composite sampler through the ice. The pH distribution of the sampled lakes is presented in the inset table. For further evidence of regional scale recovery of water quality see refs 1 and 2.

Although changes in fish predation may explain many of the changes in invertebrate populations, direct effects of water quality improvements were also detected. The number of taxa of benthic invertebrates increased from 39 in 1982/83 to 72 in 1988 (Fig. 1) and the relative abundance of many of the original species changed dramatically. Three new species of mayfly (*Hexagenia* sp., *Ephemerella* sp., *Caenis* sp.) and two species of oligochaetes (*Vejdovskyella comata, Slavina appendiculata*) appeared in the most recent survey. A single specimen of gastropoda (*Ferrissia sp.*) was also captured for the first time in 1988. Taxa scarce in the early survey, including leeches and crayfish (*Orconectes propinguus*), increased in numbers coincident with improvements in water quality. The benthic survey also indicated an increase in Iowa darters (*Etheostoma exile*), a bottom-dwelling fish. One new zooplankton species (*Epischura lacustris*) also appeared in the collections after 1981. On the basis of previous studies all the above species can be considered acid-sensitive[16–22].

Whitepine Lake is only one of many lakes in the Sudbury area where water quality has improved following reduced emissions of sulphur (Fig. 2). In January 1980 and January 1987, we sampled 66.6% ($n = 104$) of all known lake trout lakes (ranging from extinct to healthy populations) within a 1.4×10^3 km^2 area around Sudbury. The sample set was slightly biased towards larger lakes (surface area 38–13,130 ha) to permit sampling by fixed-wing aircraft. Increases in pH ($\bar{x}\Delta pH = 0.37$) and ANC ($\bar{x}\Delta ANC = 23$ μequivalent l^{-1}) occurred in 98.1 and 97.3% of the lakes, respectively. The number of lakes with pH below the threshold for reproduction of lake trout ($pH < 5.5$) declined from 45 (43.3%) to 20 (19.2%) (Fig. 2).

In summary, our data show that rapid chemical and biological recovery of industrially-acidified lakes can be accomplished simply by reducing the emission to the atmosphere of acidifying substances. Conditions suitable for the survival and reproduction of lake trout and other acid-sensitive organisms can return without liming or other ameliorative treatments. ☐

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1. Keller, W. & Pitblado, J. R. *Water Air Soil Pollut.* **29,** 285–296 (1986).
2. Dillon, P. J., Reid, R. A. & Girard, R. *Water Air Soil Pollut.* **31,** 59–66 (1986).
3. Thompson, M. E. *Water Air Soil Pollut.* **31,** 17–26 (1986).
4. Hutchinson, T. C. & Havas, M. *Water Air Soil Pollut.* **28,** 319–333 (1986).
5. Wright, R. F., Lotse, E. & Semb, A. *Nature* **334,** 670–675 (1988).

6. Forsberg, C., Morling, G. & Wetzel, R. G. *Ambio* **14,** 164–166 (1985).
7. Battarbee, R. W. *et al. Nature* **332,** 530–532 (1988).
8. Haughs, M. & Wright, R. F. Acid Deposition: *Reversibility of Soil and Water Acidification. A Review,* Air Pollution Report No. 11 (Commission of the European Communities, Brussels, 1988).
9. Schindler, D. W. *Science* **239,** 149–157 (1988).
10. Gunn, J. M. & Keller, W. *Can. J. Fish. aquat. Sci.* **41,** 319–329 (1984).
11. Gunn, J. M., McMurtry, M. J., Bowlby, J. N., Casselman, J. M. & Liimatainen, V. A. *Trans. Amer. Fish. Soc.* **116,** 618–627 (1987).
12. Beggs, G. L. & Gunn, J. M. *Water Air Soil Pollut.* **30,** 711–717 (1986).
13. Mills, K. H., Chalanchuk, S. M., Mohr, L. C. & Davies, I. J. *Can. J. Fish. aquat. Sci.* **44** (Suppl. 1), 114–125 (1987).
14. Schindler, D. W. *Reversibility of Acidification* (ed. Barth, E.) 2–13 (Elsevier Applied Science, London, 1987).
15. Gunn, J. M. *et al. Can. J. Fish. aquat. Sci.* **47,** 446–453 (1990).
16. Dermott, R. M., Kelso, J. R. M. & Douglas, A. *Water Air Soil Pollut.* **28,** 283–292 (1986).
17. Raddum, G. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 330–331 (SNSF project, 1432 As, Norway, 1980).
18. Dermott, R. M. *Hydrobiologia* **128,** 31–38 (1985).
19. Berrill, M., Hollett, L., Margosian, A. & Hudson, J. *Can. J. Zool.* **63,** 2586–2589 (1985).
20. Rahel, F. J. & Magnuson, J. J. *Can. J. Fish. aquat. Sci.* **40,** 3–9 (1983).
21. Keller, W. & Pitblado, J. R. *Water Air Soil Pollut.* **23,** 271–291 (1984).
22. Mills, K. H. & Schindler, D. W. *Water Air Soil Pollut.* **30,** 779–789 (1986).

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Tritrophic effects of a simple architectural mutation in pea plants

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WHEN studying interactions between trophic levels, ecologists often restrict their attention to two levels as a means of simplifying the analysis; unfortunately, this simplification can be misleading if tritrophic interactions (such as plant–herbivore–predator) cannot be understood by simply adding together pairwise interactions (plant–herbivore plus herbivore–predator, for example)[1,2]. We examined the significance of tritrophic interactions by asking how the morphology of the common pea (*Pisum sativum*) influences the population growth of pea aphids (*Acyrthosiphon pisum*) in the presence and in the absence of a third trophic level. We found significant interactions between the first trophic level (peas) and the third trophic level (ladybird beetles) in determining aphid population growth. Our results point out how simple genetic changes can yield morphological variants in plants that differ dramatically in their resistance to herbivores due to the effects of plant architecture on enemies of the herbivore.

Numerous studies suggest that plant morphology may indirectly influence herbivores by affecting predators or parasites[3-6]. To show that such phenomena represent tritrophic interactions, however, one needs to factor out the direct effects of plant morphology on herbivores from the indirect effects mediated by the third trophic level. We distinguished these direct and indirect effects by using a factorial design in which two different treatments of plant architecture (normal versus leafless peas) were crossed with two predator treatments (with and without ladybirds), and rates of aphid population growth were used as the response variable. A significant interaction between the predation and the plant architecture treatments would be evidence for a tritrophic interaction. Because we were also interested in the extent to which within-species variation in plant morphology shapes plant-herbivore-predator interactions, we sought architectural variants that were known to differ genetically at a limited number of loci. Previous investigations of the interplay between plant morphology and herbivore enemies have involved different species of plants[3,7,8], or different artificial substrates[9], or varieties of the same species that differ at an unknown number of unspecified genetic loci[4,5]. In contrast, we used highly backcrossed strains of peas that differed at only two loci: a normal variety and a leafless variety that is distinguished by homozygous recessive mutations at the afila and the reduced stipule loci[10].

For each experiment, individual pea plants were grown in pots 21 cm in diameter and 21 cm deep, and were enclosed in cages 32 cm tall. An excess of late instar (IV) or adult apterous aphids were first placed on each plant, and then aphids were removed until we achieved the desired initial density, which was the same for all plants within any experiment. Although we were not able to produce perfectly identical age distributions of aphids on all plants, we did not introduce any consistent biases. After the aphid populations were established, the desired number of predators (either *Coccinella septempunctata*, *Hippodamia variegata*, or *Propylea quatuordecimpunctata*) were added to plants in the 'with predator' treatment and all of the plants were caged.

Experiments were initiated with identically aged pea plants (3–5 weeks old, depending on the experiment) and were allowed to run for 2–4 days. Although 2–4 days might seem a short time over which to observe population change, it proved to be an interval over which aphid numbers changed by as much as ±100% (this is because our final census included newborn aphids).

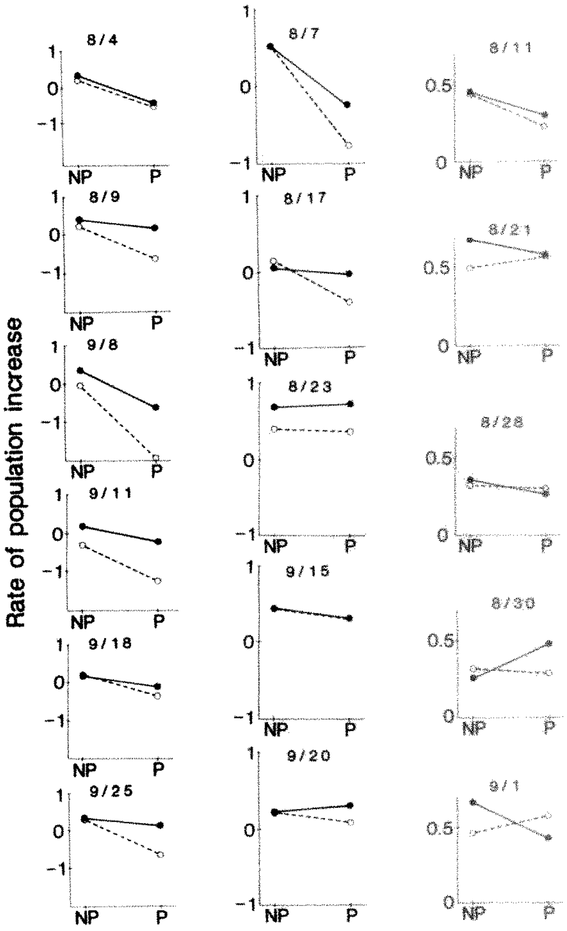

FIG. 1 Instantaneous rate of aphid population growth (per day) in factorial experiments that manipulated presence of ladybird predators (NP, no predators; P, with predators) and the architectural variety of pea. —○—, Leafless mutant; —●—, normal leafy genotype. Left column of graphs, experiments with *Coccinella septempunctata*; middle column, *Hippodamia variegata*; right column, *Propylea quatuordecimpunctata*. Each set of axes is also labelled with the date on which the experiment terminated. The decline in aphid population growth from NP to P is a measure of predator impact; the differences in slope of dashed and solid lines reflect possible interaction effects (see Table 1 for statistical significance) or 'three-trophic-level effects'. The scale is expanded fourfold for *Propylea* versus Coccinella and *Hippodamia* columns; thus the effects of *Propylea* are negligible in comparison with *Coccinella* or *Hippodamia*.

TABLE 1 Summary of two-way analyses of variance using SYSTAT MGLH (ref. 17) with balanced designs and instantaneous rate of aphid population growth as the response variable

Predator	Architecture effects	Predation effects	Interaction effect	Aphid:beetle†
Coccinella	NS	**	NS	10:2
Coccinella	**	**	**	30:2
Coccinella	**	**	**	40:3
Coccinella	**	**	NS	40:2
Coccinella	*	**	*	40:2
Coccinella	**	**	*	30:2
Hippodamia	**	**	**	10:2
Hippodamia	**	**	**	20:2
Hippodamia	**	NS	NS	30:2
Hippodamia	NS	NS	NS	20:2
Hippodamia	NS	NS	*	40:3
Propylea	NS	**	NS	20:2
Propylea	NS	NS	NS	20:4
Propylea	NS	NS	NS	30:4
Propylea	NS	NS	NS	30:7
Propylea	NS	NS	*	30:7

Each row represents an independent experiment, using different sets of plants, aphids and ladybird beetles. NS, not significant.

* $P<0.05$; ** $P<0.01$.

† Number of aphids and coccinellids initially placed on plants.

TABLE 2 The effect of pea morphology on the frequency with which foraging ladybird beetles fall off pea plants while moving in search of aphids

Ladybird species	Leafless peas	Normal peas	
Coccinella septempunctata	26 of 100 (26%)	47 of 100 (47%)	$P<0.01$
Hippodamia variegata	8 of 88 (9%)	28 of 88 (32%)	$P<0.01$

One hundred (*Coccinella*) or 88 (*Hippodamia*) individual beetles were observed for 2 min on first one pea genotype and then the other pea genotype (first genotype determined randomly). Each beetle was used only once on the two pea genotypes, which justifies analysing the results as independent trials. Observations were spread over two weeks (7–23 July) in 1988 and took place on peas planted in the ground as opposed to pots. We used a log likelihood G-test to determine whether the fraction of ladybird beetles that fell off normal peas was different from the fraction that fell off leafless peas during the brief 2-min observations.

To analyse our results we focused on the instantaneous rate of aphid population growth defined by $\ln(N_f/N_0)/t$, where N_f is the final number of aphids on the pea plant, N_0 is the initial number of aphids on the pea plant, and t is the number of days for which the experiment had been run. Using a two-way analysis of variance we asked whether there were significant effects due to the predator treatments, the plant architecture treatments, or an interaction between predation and architecture treatments. When both main effects were significant, there was generally a significant interaction between the predation and architecture treatments (in four of six occasions, Table 1). If we discount *Propylea*, which had difficulty capturing and eating pea aphids, there was a striking trend for predators to be more effective at controlling aphid populations on leafless as opposed to normal peas (in 9 of the 11 comparisons involving either *Hippodamia* or *Coccinella*, Fig. 1). Aphids better escape predation by coccinellids on normal compared with leafless peas simply because ladybirds fall off the normal variety nearly twice as frequently as they do the leafless variety (Table 2); direct observation suggests this is because the beetles can cling to stems and tendrils but have difficulty maintaining a secure grasp on smooth, slippery leaves.

Our experiments show that we may be unable to detect the importance of plant morphological variation with respect to herbivory unless a third trophic level is included in the analysis. In the absence of a third trophic level, we found that aphid population growth was only slightly reduced (on average less than 10%) on a mutant variety of peas compared with the normal genotype; however, in the presence of a third trophic level, a simple mutation to the leafless form produced peas that enjoyed greatly reduced rates of aphid population growth (on average greater than a 50% reduction) when compared with the normal genotype. Field observations using these same varieties of peas and suite of predators indicate that the interaction between plant architecture and a third trophic level is even more striking when the ladybirds roam freely from plant to plant as opposed to being confined in a cage[12]. Since plant morphology often influences predator and parasite searching patterns[3-5,7,8,11,13-15], consideration of the third trophic level needs to be incorporated into breeding programs aimed at producing resistant morphological varieties of crops[5]. Moreover, in natural plant-insect associations, there is clearly the opportunity for subtle changes in plant architecture dramatically to alter the outcome of plant-herbivore interactions because of effects at the third trophic level. □

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1. Lawton, J. & McNeill, S. *Symp. Brit. Ecol. Soc.* **20,** 223–244 (1979).
2. Price, P. *et al. A. Rev. Ecol. Syst.* **76,** 456–462 (1983).
3. Hulspas-Jordan, P. & Van Lenteren, J. *Med. Fac. Landbouw. Rijksuniv. Gent* **43,** 431–440 (1978).
4. Kauffman, W. C. & Kennedy, G. G. *Envir. Ent.* **18,** 698–704 (1989).
5. Obrycki, J. J., Tauber, M. J. & Tingey, W. M. *J. econ. Ent.* **76,** 456–462 (1983).
6. Southwood, T. R. E. in *Insects and the Plant Surface* (eds Juniper, B. & Southwood, T. R. E.) 1–22 (Edward Arnold, London, 1986).
7. Carter, M. C., Sutherland, D. & Dixon, A. F. G. *Oecologia* **63,** 394–397 (1984).
8. Evans, E. W. *N.Y. ent. Soc.* **XC,** 129–133 (1982).
9. Andow, D. L. & Prokrym, D. R. *Oecologia* **82,** 162–165 (1990).
10. Wehner, T. C. & Gritton, E. T. *J. Am. Soc. hort. Sci.* **106,** 272–278 (1981).
11. Belcher, D. W. & Thurston, R. *Envir. Ent.* **11,** 91–94 (1982).
12. Kareiva, P. in *Critical Issues in Biological Control* (eds Mackauer, E., Ehler, L. & Roland, J.) 213–238 (Intercept, Andover (UK), 1990).
13. Quilici, S. & Iperti, G. in *Ecology of Aphidophaga* (ed. Hodek, I.) 99–106 (Academia, Prague/Dr W. Junk, Dordrecht, 1986).
14. Rotheray, G. E. in *Ecology of Aphidophaga* (ed. Hodek, I.) 107–111 (Academia, Prague/Dr W. Junk, Dordrecht, 1986).
15. Shahjahan, M. & Streams, F. A. *Envir. Ent.* **2,** 921–925 (1973).
16. Bernays, E. & Graham, M. *Ecology* **69,** 886–892 (1988).
17. Wilkinson, L. Systat, the System for Statistics (SYSTAT Inc., Evanston, 1989).

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Horizontal and vertical components of head movement are controlled by distinct neural circuits in the barn owl

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To generate behaviour, the brain must transform sensory information into signals that are appropriate to control movement. Sensory and motor coordinate frames are fundamentally different, however: sensory coordinates are based on the spatiotemporal patterns of activity arising from the various sense organs, whereas motor coordinates are based on the pulling directions of muscles or groups of muscles. Results from psychophysical experiments suggest that in the process of transforming sensory information into motor control signals, the brain encodes movements in abstract or extrinsic coordinate frames[1-5], that is ones not closely related to the geometry of the sensory apparatus or of the skeletomusculature. Here we show that an abstract code underlies movements of the head by the barn owl. Specifically, the data show that subsequent to the retinotopic code for space in the optic tectum yet before the motor neuron code for muscle tensions there exists a code for head movement in which upward, downward, leftward

and rightward components of movement are controlled by four functionally distinct neural circuits. Such independent coding of orthogonal components of movement may be a common intermediate step in the transformation of sensation into behaviour.

Electrical microstimulation of the owl's optic tectum elicits a quick (saccadic), orienting movement of the head. These movements are similar to those evoked by tectal stimulation in other species[6-10] in that the magnitude and direction (vector) of the movement varies systematically with the site of stimulation revealing a topographic code for movement vector[11]. In analysing the transformation of the tectal motor code into neck motor neuron control signals, we exploited an experimental paradigm, developed by Robinson[12], that takes advantage of the fact that the post-tectal circuitry enters a brief refractory state after being activated. This refractory phenomenon is most clearly demonstrated when a pair of stimulus pulse trains is applied to a single site in the tectum. Two short (40 ms) pulse trains separated by more than 200 ms (interval between train onsets) elicit two similar movements of the head each lasting about 50 ms. But when these pulse trains are delivered at shorter intervals, the movement in response to the second pulse train is reduced and, at intervals of less than 100 ms, may completely disappear. The absence of movement in response to the second pulse train is not due simply to fatigue of tectal output neurons or of motor neurons because continuous stimulation of a site for 80 ms elicits a much larger movement that lasts for about 80 ms[11].

Although the basis of the refractory effect itself is not understood, we used it to explore the functional organization of the motor control circuitry intermediate to the tectum and spinal cord. When we stimulated two different tectal sites in rapid succession, instead of the second movement disappearing, its direction was systematically altered. For example, in the case shown in Fig. 1*a*, the first and second movements when separated by a long interstimulus interval (500 ms) were to the left-up and right-up directions, respectively. As the interval decreased from 500 to 100 ms, however, the direction of the second movement became progressively more rightward (this change in direction is opposite to that expected if the second movement were being partially averaged with the first). Intervals of 100 to 60 ms (the shortest interval used) elicited a second movement whose final position was due right of its starting position. In this case, the upward component of movement was shared by the first and second movements, and it was the upward component that was lost from the second movement, resulting in a purely rightward movement. Thus, the neural circuitry that generates saccadic movements does not become refractory in its entirety. Rather, circuits controlling upward movement can be made refractory independently of circuits controlling rightward movement.

Similarly, circuits that control leftward and upward components of movement were separated by simply reversing the order of stimulation at the same two tectal sites (Fig. 1*b*). At intervals of less than 100 ms, the direction of the second movement became purely leftward. Analogous results were obtained for paired stimulation sites that evoked movements having a common rightward component (for example, right-up and right-down; Fig. 1*c*, *d*): At intervals of less than 100 ms only the downward or upward component remained.

In all of the above cases (29 combinations in 4 owls), the direction of the second movement that remained at short intervals was very close to either a vertical or a horizontal direction. We tested whether these particular directions were dependent on the specific directions of the first and second movements, as

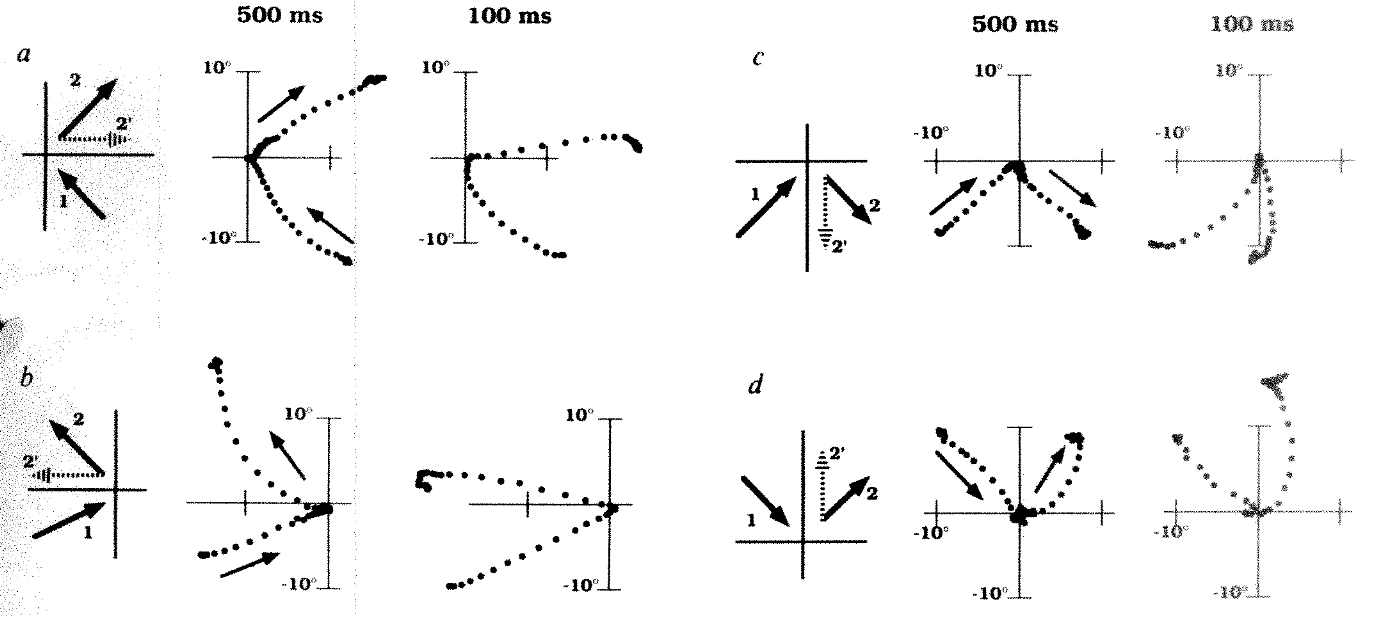

FIG. 1 Examples of head movements elicited by stimulating pairs of tectal sites sequentially at different stimulus intervals. The experimental paradigm was the same as that used by Robinson[12]. Each dot represents the position of the head at 5 ms intervals. The axes represent the degree of rotation of the head in the vertical (elevational) and horizontal (azimuthal) directions. Movements were translated according to the position of the head following the first movement; left is left, up is up, etc. *a*), A left-up first movement (1) followed by a right-up second movement at stimulus onset intervals of 500 (2) and 100 (2') ms. At 100 ms, only the rightward component of the second movement persists. *b*, A right-up movement (1) followed by a left-up movement at the same two stimulus onset intervals. At 100 ms, only the leftward component of the second movement persists (2'). *c*, A right-up movement (1) followed by a right-down movement (2 and 2'). *d*, A right-down movement (1) followed by a right-up movement (2 and 2').

METHODS. Owls were prepared for chronic electrode implant under ketamine (10 mg $\mathrm{kg}^{-1}\,\mathrm{h}^{-1}$) and diazepam (0.6 mg $\mathrm{kg}^{-1}\,\mathrm{h}^{-1}$) anaesthesia. Epoxy-coated tungsten electodes (tip diameter at base 125 μm, exposed tip length 250 μm) plated at the tip with gold and/or platinum (impedance at 1 kHz = 10 kΩ) were lowered into the optic tectum. Visual receptive fields were measured to identify the location of the electrode in the tectum and the shank was cemented to the skull. Four to six electrodes were placed in the two tectal lobes representing sensory field locations (and movement directions) in all spatial quadrants. Several days later the owls were placed in a tube which restricted limb movements but allowed for free head movements. They were positioned at the centre of the induction coils of a 6-ft search coil system (CNC Engineering). Electrical stimulation (constant current, electrically isolated 300 μs cathodal pulses, 75-240 μA, 40-60 ms pulse trains at 300 Hz) was applied through the microelectrodes. The resulting movements were measured from the output of a 2 cm diameter search coil that was attached to the skull. The output of the search coil was recorded, calibrated, and displayed online with a Macintosh II computer, and saved on magnetic disk for subsequent quantitative analysis.

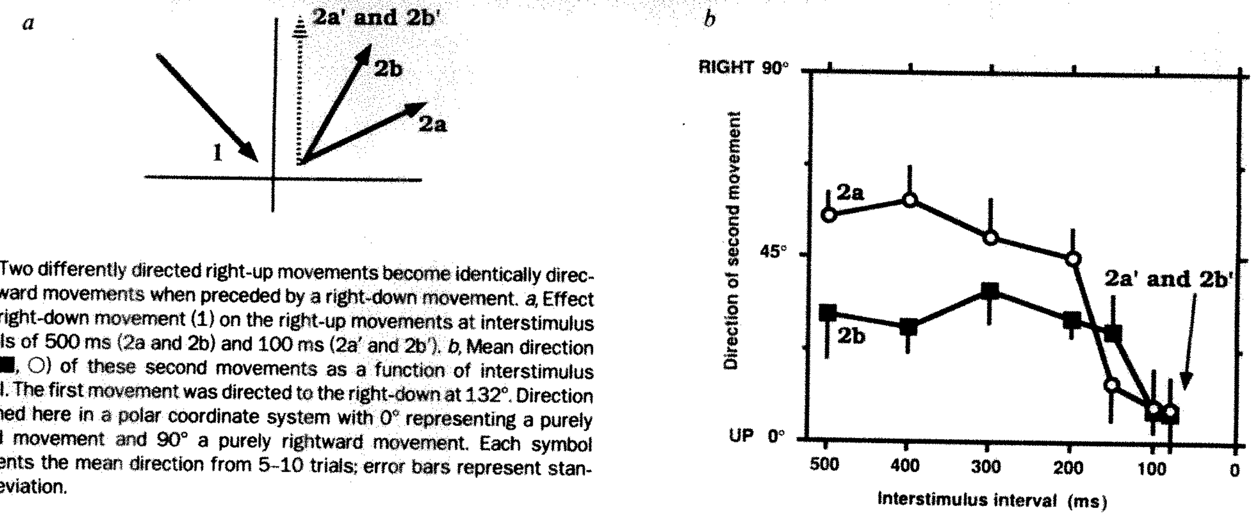

FIG. 2 Two differently directed right-up movements become identically directed upward movements when preceded by a right-down movement. *a*, Effect of the right-down movement (1) on the right-up movements at interstimulus intervals of 500 ms (2a and 2b) and 100 ms (2a' and 2b'). *b*, Mean direction plots (■, ○) of these second movements as a function of interstimulus interval. The first movement was directed to the right-down at 132°. Direction is defined here in a polar coordinate system with 0° representing a purely upward movement and 90° a purely rightward movement. Each symbol represents the mean direction from 5–10 trials; error bars represent standard deviation.

would be predicted by vector addition or by post-tectal circuitry that lacked discrete directional components. To examine this issue, we measured the effect of a single first movement (right-down; movement 1 in Fig. 2*a*) on two differently directed second movements both into the right-up quadrant of space (movements 2a and 2b in Fig. 2). When either of the two right-up movements was preceded by a movement to the right-down, they changed to become identical, purely upward movements. The reverse strategy yielded the same result: when a single second movement (such as left-down) was tested against two differently directed first movements into a given quadrant of space (such as left-up), the direction of the second movement at short intervals was always purely downward. The same result was observed in each of seven sets of movement combinations. Thus, for movements directed into a given quadrant, the alignment of the refractory movement with one of the orthogonal axes was independent of the direction of the non-refractory movement. These results are incompatible with vector addition, vector averaging, or other models that represent movement direction as a continuous function.

A different phenomenon was observed when two evoked movements were in opposite directions. Under these conditions, the second movement continued to be expressed regardless of the interval. Although intervals of less than 100 ms sometimes caused the first movement to be interrupted by the initiation of the second, the direction of the second movement was unaltered. Thus, the neural circuits that generate saccades into opposite quadrants of space do not cause each other to become refractory, indicating that they can operate independently of each other.

These results suggest that movement direction is encoded by the coordinated action of a small number of distinct neural circuits (referred to henceforward as saccade generators), each controlling movement in a particular direction. We determined the number of saccade generators and the directions they encode by examining the effect of one movement on another for many combinations of movement directions. Whenever the two movements were into adjacent quadrants of space, the direction of the second movement at short intervals was altered predictably to one of four directions: upward, downward, leftward, or rightward (Fig. 3). These results suggest that the tectal command for movement accesses four independent saccade generators, each responsible for the component of a movement in one of these four orthogonal directions.

The direction of movement evoked by stimulation in one tectal lobe could be altered equally well by stimulation in the same (Fig. 1*c*, *d*) or in the opposite tectal lobe (Fig. 1*a*, *b*). This implies that the saccade generators are located outside the tectum and that both tectal lobes access the same generators. The existence of a short delay (<20 ms) between tectal stimulation and the onset of movement, combined with the anatomical observations

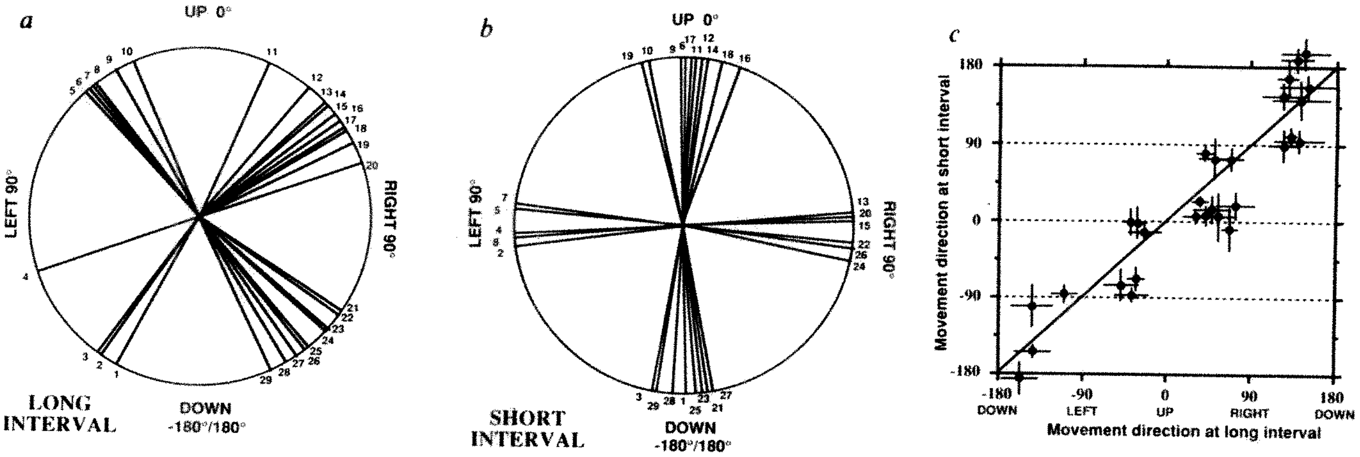

FIG. 3 Summary diagram of the directions of movements elicited from all tectal sites tested when preceded at (*a*) long (500 ms) and (*b*) short latency (60–100 ms) by a movement into an adjacent quadrant. Movement direction is represented as a unit vector representing the mean direction of 5–10 movements. Unit vectors are calculated as the direction from starting to the final resting head position: up is up, left is left, etc. Each case is numbered such that, for example, 1 in (*a*) represents a movement elicited from the same stimulus site as 1 in (*b*). *c*, Standard deviations (error bars) associated with the mean movement directions under each condition given in *a* and *b*. The diagonal line represents the hypothesis that the second movement was not affected by the first. The plot also shows that movements in each of the non-orthogonal directions can be altered to either of the two component orthogonal directions.

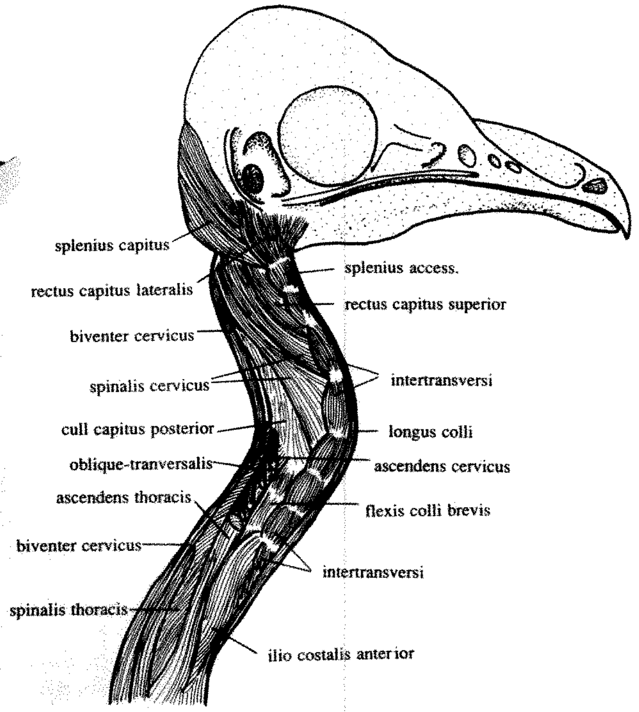

FIG. 4 Diagram of the superficial musculature of the neck of the barn owl (*Tyto alba*). The neck is S-shaped, contains 13 vertebrae and at least 31 distinct muscle pairs, some of which are segmentally arranged whereas others span multiple segments.

on tecto-spinal relay sites in the brainstem tegmentum[13-15], suggest that the saccade generators are located in brainstem tegmental regions.

The existence of separate circuits for controlling horizontal and vertical components of movement in a complex skeleto-muscular system indicates that such an intermediate representation may be a general principle of sensorimotor organization. In the case of eye movements, the similar orthogonal character of the saccade generators[12] may be related to the essentially orthogonal arrangement of the extraocular rectus muscles. In contrast, head movements depend on the action of many pairs of muscles pulling in a variety of directions, and there is no apparent organization of muscle-pulling directions according to orthogonal planes of movement (Fig. 4). Thus, unlike in eye motor control, the independent coding of orthogonal movement components of the head is markedly different from both sensory and the muscle coordinate frames, implying that it operates as an abstract system intermediate to the sensory and motor processes that control orienting movements.

These observations, together with observations in other systems, suggest that orthogonal coordinate systems may be a general property of intermediate stages in motor control hierarchies. In cats and monkeys, electrical stimulation of several brainstem tegmental regions elicits either primarily horizontal or vertical head movements[16,17]. Pyschophysical experiments on human arm and hand movments suggest that they also may be encoded in terms of their azimuthal and elevational components[5,18,19]. Lesion studies in the frog have demonstrated that leftward and rightward horizontal components of whole-body orienting movements are controlled by distinct circuitry, and that these circuits are independent of those controlling elevation and distance[20-23]. This evidence suggests that, despite the differences in the skeletal and muscular organization among head, arm and body movements, there may be for each an intermediate representation of movement direction encoded in orthogonal spatial coordinates.

There may be several advantages to representing movements in orthogonal directional components. The orthogonal coordinate system facilitates the integration of spatial information coming from different sensory modalities, allowing several sensory systems to access a single motor command structure[18,24-26]. In addition, it facilitates the coordination of movements by different body parts when the goal of a movement can be accomplished in a variety of ways. For example, redirection of gaze can be carried out by movements of the eyes, head or body or by any combination of these three. To locate a desired target accurately, information about the change in gaze contributed by each kind of movement must be combined in a common form before being compared with the change in gaze that was intended. Representing orienting movements in terms of their horizontal and vertical components may provide such a common coordinate frame for feedback regulation of movements by multiple body parts. ☐

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1. Bernstein, N. *The Co-ordination and Regulation of Movements* (Pergamon, Oxford, 1967).
2. Hollerbach, J. M. & Flash, T. *Biol. Cybern.* **44,** 67–77 (1982).
3. Morasso, P. *Expl. Brain Res.* **42,** 223–237 (1981).
4. Bizzi, E., Accornero, N., Chapple, W. & Hogan, N. *J. Neurosci.* **4,** 2738–2744 (1984).
5. Soechting, J. F. & Flanders M. *J. Neurophysiol.* **62,** 595–608 (1989).
6. Syka, J. & Radil-Weiss, T. *Brain Res.* **28,** 567–572 (1971).
7. Ewert, J.-P. *Brain Behav. Evol.* **3,** 36–56 (1970).
8. Schiller, P. J. & Stryker, M. *J. Neurophysiol.* **35,** 915–924 (1972).
9. Wurtz, R. H. & Goldberg, M. E. *Science* **171,** 82–84 (1971).
10. Sparks, D. L. *Brain Res.* **156,** 1–16 (1978).
11. du Lac, S. & Knudsen, E. I. *J. Neurophysiol.* **63,** 131–146 (1989).
12. Robinson, D. *Vis. Res.* **12,** 1795–1808 (1972).
13. Masino, T. & Knudsen, E. I. *Soc. Neurosci. Abstr.* **14,** 1236 (1988).
14. Masino, T. & Grobstein, P. *J. comp. Neurol.* **291,** 103–127 (1990).
15. Grantyn, A. & Berthoz, A. in *Control of Head Movement* (eds Peterson B. & Richmond, F.J.) 224–244 (Oxford University Press, 1988).
16. Fukushima, K. *Prog. Neurobiol.* **29,** 107–192 (1987).
17. Westheimer, G. & Blair, S. M. *Expl Brain Res.* **24,** 89–95 (1975).
18. Soechting, J. F. & Ross, B. *Neuroscience* **13,** 595–604 (1984).
19. Lacquaniti, F. *Trends Neurosci.* **12;** 287–291 (1989).
20. Kostyk, S. K. & Grobstein, P. *Neuroscience* **21;** 41–55 (1987).
21. Grobstein, P. *Brain Behav. Evol.* **31,** 34–48 (1988).
22. Masino, T. & Grobstein, P. *Expl. Brain Res.* **75,** 227–244 (1989).
23. Masino, T. & Grobstein, P. *Expl. Brain Res.* **75,** 245–264 (1989).
24. Knudsen, E. I., du Lac, S. & Esterly, S. *A. Rev. Neurosci.* **10,** 41–65 (1987).
25. Simpson, J. I. & Graf, W. *Rev. Oculomotor Res.* **1,** 3–20 (1985).
26. Grobstein, P. *Visuomotor Coordination* (eds Ewert, J.-P. & Arbib, M. A.) 537–568 (Plenum, New York, 1989).

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Odour-modulated collective network oscillations of olfactory interneurons in a terrestrial mollusc

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DETERMINATION of the dynamical structure of neural circuits—the general principles of how neural activity varies with time and manipulates information—is a prerequisite to understanding their computational function[1]. Rhythmically active or oscillating neural circuits are particularly interesting dynamical structures, as rhythms and oscillations are a prominent feature of mammalian central nervous system electrophysiology. Coherent oscillations by networks of interneurons are observed in the vertebrate olfactory system[2,3] and have recently been described in mammalian visual cortex[4-6]. These interneuronal networks display oscillations in local field potential (LFP) and probability of producing action potentials that are highly correlated between subcircuits sharing

the same stimulus features. Much less is known about the existence and importance of network oscillations in the higher centres of invertebrates[7]. Here we report that a network of olfactory interneurons in the cerebral ganglion of the terrestrial mollusc *Limax maximus* also displays coherent oscillations in LFP which are modified by odour input. This dynamical structure could be central to the odour recognition and odour learning ability of *Limax*[8,9].

The network of olfactory interneurons we studied is known as the procerebral (PC) lobe, which contains about 50% of all neurons (10^4 or 10^5 cells) in the entire *Limax* nervous system. The PC lobe receives odour information directly from the superior and inferior noses. Its anatomical features[10-13] suggest substantial feedback synaptic connections. Extracellular recordings (0-3 Hz bandwidth) from the procerebral lobe of *Limax* (Fig. 1) reveal a periodic potential with a fundamental frequency of 0.69 ± 0.12 Hz ($n = 62$) (Fig. 2*a*). The LFP oscillation is produced by a mechanism intrinsic to the PC lobe, based on the observations that the oscillation is only recorded by an electrode in the PC lobe and that a PC lobe completely isolated from the rest of the cerebral ganglion still exhibits the oscillation (Fig. 2*b*). An oscillating LFP is recorded from any point within the PC lobe. Dual microelectrode recordings from within the same PC lobe demonstrate that the oscillations are always phase-locked. Apical and basal recordings within the lobe are in phase but of opposite polarity, whereas the intermediate central region shows a biphasic potential (Fig. 2*c*). Because the apical and basal regions of the PC lobe show LFP changes of opposite sign, current will flow between them on a cycle by cycle basis, and some of this intra-PC lobe current flow can be sensed by a surface suction electrode (Fig. 2*d*), directly analogous to surface EEG recordings from mammalian olfactory bulb.

Intracellular recordings from *Limax* PC lobe interneurons demonstrate periodic oscillations of membrane potential (Fig. 2*e*) with oscillation frequency similar to that of the LFP. During the depolarizing phase of these oscillations, cells can remain silent or can fire one or a short burst of action potentials. Thus the oscillations in *Limax* PC lobe LFP have a correlate in membrane potential oscillations. Extracellular recordings demonstrate that all spike activity that can be recorded in the PC is phase locked to the LFP oscillation (Fig. 3*a*, inset). Over 90% of the action potentials occur in the 500 ms preceding the peak of the LFP oscillation (Fig. 3*a*). Although the ionic currents underlying the LFP and the precise relationship of the LFP to the spike and synaptic activity is at present unclear, the results suggest that the LFP is produced by synaptically-dependent events set up by the coherent collective activity of PC lobe interneurons.

Activation of olfactory afferents by odours under natural conditions will produce input to the PC lobe over several cycles of the LFP oscillation. To determine how the temporal relationship between afferent fibre activation and the LFP oscillation affects the synaptic response produced, the sizes of synaptically dependent evoked potentials were measured for olfactory nerve (ON) shock given at different phases of the LFP oscillation. ON shock produces a two-component response in the neuropil of the PC lobe (Fig. 3*b*, inset). The first peak is due to the action potentials in the afferent fibres entering the lobe from the ON. The second slower peak is due to synaptic events elicited by the afferent volley[9]. The size of the synaptic response to ON shock is dependent on the time of arrival of the afferent volley relative to the phase of the endogenously oscillating LFP (Fig. 3*b*). Thus the postsynaptic effects of afferent fibre activation by odour will vary depending upon the phase of the intrinsic PC lobe oscillation at which it arrives. ON shock also transiently alters the PC oscillation frequency and waveform for 5-15 s after the stimulus and resets the rhythm relative to its preshock phase.

The importance of the intrinsic oscillation of the PC lobe in the processing of olfactory information is suggested by experiments using a preparation which retained the olfactory epithelium of the nose connected to the PC lobe via the digitate ganglion and ON[14]. The receptor surface was in air while the remainder of the preparation remained immersed in saline. A two-channel puffer was used to apply either moist air or air equilibrated with a 10^{-5}% solution of 2-ethyl-3-methoxypyrazine (potato odour; see ref. 15) to the olfactory surface while recording *en passant* from the olfactory nerve and from the entire PC

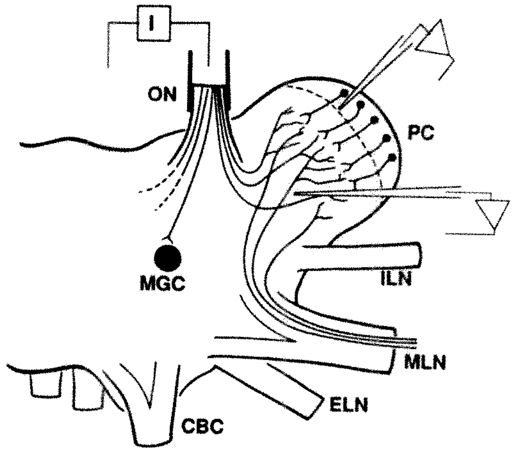

FIG. 1 Schematic diagram of the cerebral ganglion of *Limax maximus*. The olfactory nerve (ON) is in a suction electrode for stimulation. Extracellular micropipette electrodes are shown recording from apical (cell body) region and basal neuropil in the procerebral (PC) lobe. Specimens of *Limax maximus* ($n=74$) were anaesthetized by chilling on ice for 30 min before the brain was removed into cold (4-6 °C) saline for isolation of the cerebral ganglia. Isolated PC lobe preparations were made by cutting across the base of the lobe from the insertion of the ON to the insertion of the internal lip nerve (ILN). Nose-brain preparations retained the olfactory neuroepithelium and digitate ganglion from the tip of the superior tentacle connected to the ON. The PC lobe also receives input from the inferior nose via the medial lip nerve (MLN). CBC-cerebrobuccal connective, ELM-external lip nerve, MGC-metacerebral giant cell.

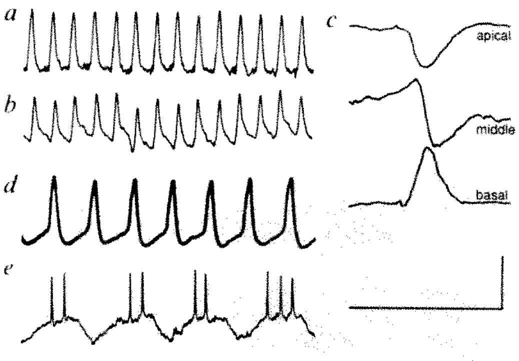

FIG. 2 Recordings of spontaneous local field potential (LFP) oscillations from a PC lobe. *a*, PC lobe attached to the cerebral ganglion as in Fig. 1. Recording site located in the neuropil. *b*, Same recording site in same lobe as (*a*) but after complete isolation from the cerebral ganglion. *c*, Simultaneous recordings from apical, central and basal recording sites in a PC lobe. *d*, Coherent PC lobe oscillation recorded from an isolated lobe lodged in a suction electrode. *e*, Intracellular recording from a neuron in the PC lobe. Calibration bars are 10 s, 1 pA (*a*, *b*); 700 ms, 3 pA (*c*); 10 s, 200 μV (*d*); 2.5 sec, 50 mV (*e*). Extracellular recordings were made either with a List model EPC 5 or a Getting model 5A amplifier and were band-pass filtered between 0-3 Hz using a PAR model 113. Data were recorded on a Gould model 220 chartwriter or digitized using a MacPacq system and stored on an Apple MacII computer for later analysis. Intracellular recordings were made using beveled glass microelectrodes of 80-120 MΩ resistance and a NeuroData IR-283 amplifier.

lobe with a suction electrode. A 10 s puff of potato odour activated many small amplitude units and a few large amplitude units in the ON and dramatically altered the waveform and frequency of the PC lobe oscillation (Fig. 4*a*). A puff of moist air at the same velocity elicited much less activity in both the ON and in the PC lobe (Fig. 4*b*). The response during odour application is predominantly a transient reduction in amplitude (15.4 ± 2.3%, $n = 14$) (mean ± s.e.m.) and reduction in frequency (9.2 ± 2.8%, $n = 15$) of the endogenous oscillation. This pattern of response was seen in 26 of 29 trials with 5 nose-brain preparations. These results indicate that activation of one of the normal sensory input pathways to the PC lobe using a behaviourally relevant stimulus at a concentration known to be attractive to the intact animal[15] produces, among other effects, transient changes in the endogenously active local circuit responsible for the oscillating LFP.

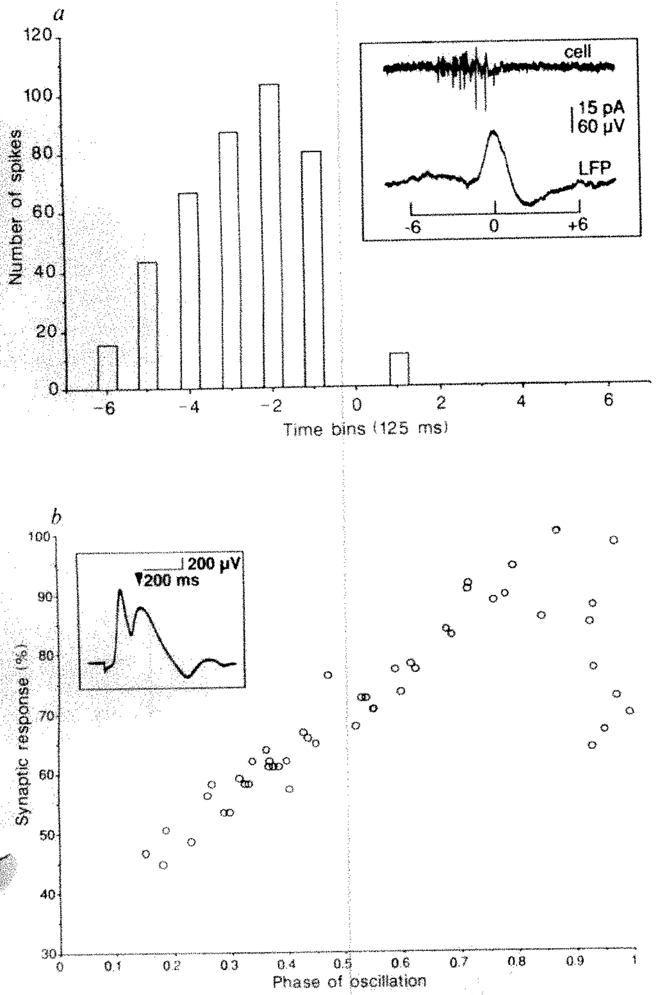

FIG. 3 Spontaneous oscillation phase data. *a*, Extracellular cell-attached patch electrode recordings ($n = 45$) showing spike activity phase-locked to the spontaneously oscillating local field potential (LFP). Inset: Typical record of multiunit extracellular recording and simultaneously recorded local field potential. Calibration bar is 15 pA for cells, 60 µV for LFP. Histogram shows number of action potentials occurring in 125 ms time bins with zero time taken at the peak of the LFP. *b*, Amplitude of the synaptic current evoked in the PC lobe by supramaximal shock of the ON depends on the phase of the spontaneous oscillation at which it is evoked. Inset: the two-phase response to a single ON shock. The second peak of the response is the calcium-sensitive synaptically-dependent event. Calibration bars indicate 150 ms, 300 µV. The graph shows that the evoked synaptic response amplitude depends on spontaneous oscillation phase. Data samples (5 s) were obtained every 100 s, with ON shock occurring 4 s into the sample. The time of the last complete oscillation cycle before the shock (T_1) was measured, as was the time from the last oscillation peak before the shock to the peak of the synaptic response (T_2). The phase of occurrence of the synaptic response was taken as (T_2/T_1). The evoked synaptically-dependent event is minimal just before and after the occurrence of the peak of the spontaneous oscillation and then increases smoothly in amplitude over the middle 80% of the inter-peak interval.

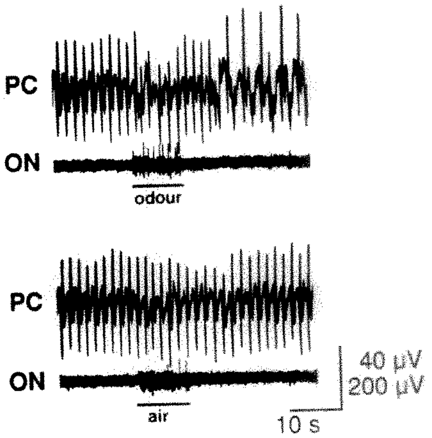

FIG. 4 Response to potato odour recorded using a nose-brain preparation. Suction electrodes recorded *en passant* from the ON and from the surface of the PC lobe. *a*, A 10-s puff of air saturated with the vapour of 10^{-5}% (v/v) of 2-ethyl-3-methoxypyrazine applied to the receptor epithelium of the nose elicited multiunit activity in the ON and dramatically altered the waveform and frequency of the PC lobe oscillation. *b*, A 10-s puff of moist air at the same velocity produced much less activity in the ON and a smaller alteration in the PC lobe LFP oscillation. The change in oscillation frequency following odour offset shown in (*a*) was not observed in every preparation.

The existence in *Limax* of the oscillating LFP and its responsivity to ON shock and odour-elicited input from the olfactory epithelium establishes yet another functional parallel between the molluscan and mammalian olfactory systems. A striking feature of mammalian olfactory bulb is the endogenously oscillating 40 Hz LFP that is enhanced by inspiration of odours[16-20]. The amplitude of the LFP oscillation varies over the bulbar surface. The spatial pattern of oscillation amplitudes could represent an odour and can be altered by conditioning[21-23]. A theoretical model has explored how the LFP oscillation by the olfactory bulb may contribute to the signal processing functions in the detection of weak odours[24]. Different models have explored how the olfactory bulb[25] or olfactory (piriform) cortex[26-28] could implement associative (content-addressable) memories. The possibility that endogenous oscillations in the second-order olfactory circuit of *Limax* allow detection of weaker odour inputs than would otherwise be the case has been supported by recent studies of LIMAX, a neural network model of the *Limax* olfactory processing and odour learning circuit[29]. With the identification of an output pathway from the PC lobe to the pedal ganglia[12] it may be possible to test this idea directly. The rhythmic synchrony of PC lobe cell activity coupled throughout the entire lobe must have a critical role in the odour processing function of this structure. The striking functional analogy between such phylogenetically disparate systems indicates that the computational use of the local circuit oscillation is fundamental to the tasks of these types of olfactory processing networks. Other olfactory systems, for example the central olfactory pathways of insects[30,31], may display similar dynamics.

The oscillating LFP in *Limax* PC lobe is modulated in both waveform and frequency by dopamine and serotonin[32] (A.G., L. Rhines, J. Flores, and D.W.T., unpublished data), both of which are present in fibres entering the lobe from sources extrinsic to the lobe[33,34]. Both serotonin, previously implicated in mechanisms of molluscan synaptic plasticity[35-39], and dopamine alter the phosphorylation state and rate of synthesis of specific proteins in neurons of the PC lobe[40,41]. Therefore, as in mammalian olfactory bulb[42-44], input pathways exist that could modify PC lobe circuitry during aversive odour conditioning. ☐

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1. Tank, D. W. *Semin. Neurosci.* **1,** 67–79 (1989).
2. Freeman, W. J. *Mass Action in the Nervous System* (Academic, New York, 1975).
3. Shepherd, G. M. in *Taste, Olfaction and the Central Nervous System* (ed. Pfaff, D. W.) 307–332 (Rockefeller University Press, New York, 1985).
4. Gray, C. M. & Singer, W. *Proc. natn. Acad. Sci. U.S.A.* **86,** 1698–1702 (1989).
5. Gray, C. M., Konig, P., Engel, A. K. & Singer, W. *Nature* **338,** 334–337 (1989).
6. Eckhorn, R. *et al. Biol. Cybern.* **60,** 121–130 (1988).
7. Bullock, T. H. & Basar, E. *Brain Res. Rev.* **13,** 57–75 (1988).
8. Gelperin, A., Tank, D. W. & Tesauro, G. in *Neural Models of Plasticity* (eds Byrne, J. H. & Berry, W. O.) 133–159 (Academic, New York, 1989).
9. Sahley, C. L. in *Connectionist Modeling and Brain Function: The Developing Interface* (eds Hanson, S. & Olson, C.) 36–73 (MIT, Cambridge, 1990).
10. McCarragher, G. & Chase, R. *J. Neurobiol.* **16,** 69–74 (1985).
11. Zs.-Nagy, I. & Sakharov, D. A. *Tissue & Cell* **2,** 399–411 (1970).
12. Chase, R. & Tolloczko, B. *J. comp. Neurol.* **283,** 143–152 (1989).
13. Veratti, E. *Mem. Real. Inst. Lomb. Sci. Lett.* **18,** 163–179 (1900).
14. Egan, M. E. & Gelperin, A. *J. mollusc. Stud.* **47,** 80–88 (1981).
15. Hopfield, J. F. & Gelperin, A. *Behav. Neurosci.* **103,** 329–333 (1989).
16. Freeman, W. J. *Biol. Cybern.* **35,** 221–234 (1979).
17. Adrian, E. D. *J. Physiol.* **100,** 459–473 (1942).
18. Adrian, E. D. *Electroenceph. clin. Neurophysiol.* **2,** 377–388 (1950).
19. Adrian, E. D. *Br. med. Bull.* **6,** 330–333 (1950).
20. Freeman, W. J. & Skarda, C. A. *Brain Res. Rev.* **10,** 147–175 (1985).
21. Gray, C. M., Freeman, W. J. & Skinner, J. E. *Behav. Neurosci.* **100,** 585–596 (1986).
22. Freeman, W. J. & Viana Di Prisco, G. *Behav. Neurosci.* **100,** 753–763 (1986).
23. Baird, B. *Physica* **22D,** 150–175 (1986).
24. Li, Z. & Hopfield, J. J. *Biol. Cybern.* **61,** 379–392 (1989).
25. Freeman, W. J., Yao, Y. & Burke, B. *Neur. Net.* **1,** 277–288 (1988).
26. Wilson, M. A. & Bower, J. M. in *Neural Information Processing Systems* (ed. Anderson, D. Z.) 114–126 (American Institute of Physics, New York, 1988).
27. Lynch, G., Granger, R., Baudry, M. & Larson, J. in *Neural Connections, Mental Computation* (eds Nadel, L., Cooper, L., Culicover, P. & Harnish, R. M.) 247–289 (MIT Press, Cambridge, 1988).
28. Haberly, L. B. *Chem. Senses* **10,** 219–238 (1985).
29. Hopfield, J. J. in *Computer Simulation in Brain Science* (ed. Cotterill, R. M. J.) 405–415 (Cambridge University Press, New York, 1988).
30. Homberg, U., Christensen, T. A. & Hildebrand, J. G. *A. Rev. Ent.* **34,** 477–501 (1989).
31. Menzel, R. in *Perspectives In Neural Systems and Behavior* (eds Carew, T. & Kelley, D.) 249–266 (A. R. Liss, New York, 1989).
32. Rhines, L. thesis, Princeton Univ. (1989).
33. Osborne, N. N. & Cottrell, G. A. *Z. Zellforsch. mitrosk. Anat.* **112,** 15–30 (1971).
34. Yamane, T., Gelperin, A. & Delaney, K. *Soc. Neurosci. Abstr.* **12,** 862 (1986).
35. Kandel, E. R. & Schwartz, J. H. *Science* **218,** 433–443 (1982).
36. Sweatt, J. D. & Kandel, E. R. *Nature* **339,** 51–54 (1989).
37. Crow, T. *Trends Neurosci.* **11,** 136–142 (1988).
38. Eskin, A., Garcia, K. S. & Byrne, J. H. *Proc. natn. Acad. Sci. U.S.A.* **86,** 2458–2462 (1989).
39. Grover, L. M., Farley, J. & Auerbach, S. B. *Brain Res. Bull.* **22,** 363–372 (1989).
40. Yamane, T. & Gelperin, A. *Cell. mol. Neurobiol.* **7,** 291–301 (1987).
41. Yamane, T., Oestreicher, A. B. & Gelperin, A. *Cell. mol. Neurobiol.* **9,** 447–459 (1989).
42. Macrides, F., Davis, B. J., Young, W. M., Nadi, N. S. & Margolis, F. L. *J. comp. Neurol.* **203,** 495–514 (1981).
43. Shipley, M. T., Halloran, F. J. & de la Torre, J. *Brain Res.* **329,** 294–299 (1985).
44. McLean, J. H. & Shipley, M. T. *J. Neurosci.* **7,** 3016–3028 (1987).

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Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy

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THE period of natural cell death in the development of rodent motor neurons is followed by a period of sensitivity to axonal injury[1-3]. In the rat this early postnatal period of vulnerability coincides with that of very low ciliary neurotrophic factor (CNTF) levels in the sciatic nerve before CNTF increases to the high, adult levels[4]. The developmental time course of CNTF expression, its regional tissue distribution and its cytosolic localization (as suggested by its primary structure)[4,5] favour a role for CNTF as a lesion factor rather than a target-derived neurotrophic molecule like nerve growth factor. Nevertheless CNTF exhibits neurotrophic activity *in vitro* on different populations of embryonic neurons[6]. To determine whether the vulnerability of motor neurons to axotomy in the early postnatal phase is due to insufficient availability of CNTF, we transected the axons of newborn rat motor neurons and demonstrated that local application of CNTF prevents the degeneration of the corresponding cell bodies.

We have chosen the rat facial nerve as an experimental system as it exclusively contains motor axons distal to the stylomastoid foramen. The corresponding cell bodies comprise a homogeneous, well-delineated nucleus in the ventral-lateral region of the brain stem (Fig. 1). Moreover, the retrograde and degenerative changes occurring after facial-nerve lesion have been investigated in great detail[7]. The facial nerves of newborn rats were unilaterally transected on the first postnatal day (see Table 1) and morphology of the facial nuclei was analysed 1 week after lesion. More than 80% of the cell bodies on the side having the lesion were completely lost after 1 week (Table 1). The remaining motor neurons showed severe signs of degeneration[8], that is, the nuclei were located eccentrically and the Nissl bodies showed the characteristic dispersion that occurs in adult motor neurons after axotomy (Fig. 1). In adult animals astrocytes react characteristically to peripheral axonal lesion of motor neurons by increasing their glial fibrillary acidic protein (GFAP) production[9,10]. In agreement with this there was enhanced GFAP staining in the astrocytes surrounding the motor neurons on the side having the lesion (data not shown). This is noteworthy because, at the end of the first postnatal week there is normally little GFAP reactivity in the brain stem; it only becomes weakly apparent at later developmental stages[11].

TABLE 1 Motor neuron survival after lesion of the facial nerve in newborn rats

	Number of surviving neurons ± s.e.m. Side with lesion	Number of surviving neurons ± s.e.m. Side without lesion
Untreated animals ($n=1$)	685	2,985
Animals treated with BSA gel foam ($n=3$)	620 ± 98*	3,271 ± 61
Animals treated with gel foam containing 5 μg of CNTF ($n=4$)	2,503 ± 487*	3,281 ± 112

Newborn Wistar rats were anaesthetized by hypothermia and the right facial nerves cut ~1 mm distal to the stylomastoid foramen. Gel foam (Spongostan, gift of K. Unsicker, Marburg) was cut into small pieces (3 mm long) and soaked in PBS buffer (20 μl) containing 5 μg purified adult rat sciatic nerve CNTF[4,13]. The soaked foam pieces were inserted at the sites of lesion. Control animals were treated with gel foam soaked in PBS buffer containing 5 μg BSA (Fraction V, Sigma) (20 μl). The skin was sutured with silk (Ethicon 3-0) and the animals returned to their mother. Operated rats were inspected daily, lesion of the right facial nerve was detected in the animals by lack of movement of the right whisker and the right corner of the mouth. On postnatal day 7 the animals were anesthetized with ether and killed by transcardial perfusion with 4% formaldehyde. The brainstem was dissected, postfixed (1 h), rinsed in water, dehydrated with increasing concentrations of ethanol (70–100%) and embedded in paraffin. Serial sections 7 μm were made from the whole brainstem. After Nissl staining the motorneurons of both facial nuclei were clearly detectable. Only cells containing a clearly-visible nucleolus were counted, in every fifth section, as described previously[14].

* $P<0.05$, t-test

The application of CNTF to the proximal stump of the nerve in which the lesion had been made (5 μg of highly purified CNTF[4] corresponding to 50,000 TU of biological activity) resulted in the survival of most of the motor neurons up to 1 week after lesion (Table 1). Morphological degenerative changes in

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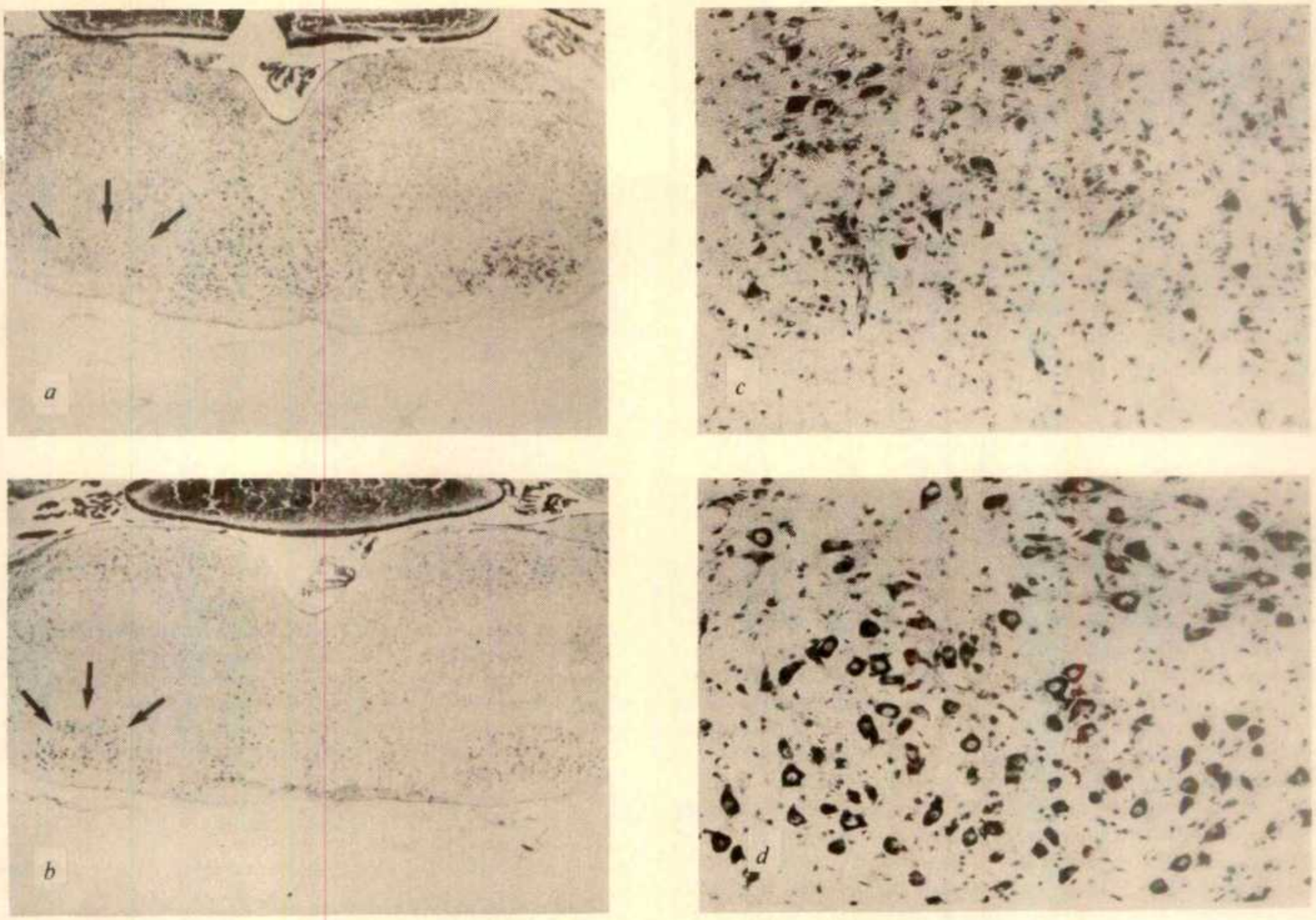

FIG. 1 *a*, *b*, Sections of the brainstem of 7-day-old rats: The facial nucleus of the side without a lesion is located on the righthand side. The facial nucleus containing the cell bodies of the neurons in which lesions have been made are located on the lefthand side (arrows). *a*, Control animal after insertion of gel foam containing 5 μg of BSA. *b*, CNTF-treated animal. (Magnification ×25). c, d, Cell bodies of the facial nucleus seven days after lesion. *c*, Control animal treated with BSA. *d*, CNTF-treated animal. (Magnification ×250). The nuclei of the motor neurons in which a lesion has been made in BSA-treated animals (*c*) are deformed and located peripherally (a typical sign of chromatolysis), whereas in CNTF-treated animals most nuclei appear round in shape and are located in the centre of the cell (*d*).

facial neurons in which a lesion had been made were also markedly reduced compared to those observed in the small number of surviving neurons where the lesion had been made and the animals BSA-treated (Fig. 1*c*,*d*). Despite the survival of most motor neurons in which a lesion had been made, there was still an enhanced GFAP reactivity in the facial nucleus of CNTF-treated nerves (data not shown), showing that CNTF can prevent the degenerative changes in motor neurons (although the reactive changes in astrocytes were still clearly visible). Immunohistochemical procedures[12] did not show whether there was a quantitative difference in the GFAP staining between the CNTF- and BSA-treated animals.

These experiments show that the local application of purified CNTF prevents the lesion-induced death of motor neurons in the rat facial brain stem nucleus. This observation is consistent with our hypothesis that in adult animals the CNTF—present in large quantities in non-neuronal cells of the peripheral nerves—is released after lesion in quantities sufficient to prevent the degeneration of motor neurons in which a lesion has been made. In contrast, in the early postnatal period the quantities of CNTF available are normally insufficient to prevent this, unless exogenous CNTF is applied locally. Although our experiments do not indicate whether the protective effects of locally applied CNTF are due to direct or indirect action on motor neurons, CNTF does support the survival of motor neurons during the naturally occurring period of cell death (Arakawa and M.S., unpublished observation). Thus, CNTF may act as a general neuronal-survival factor unrestricted in its time of action.

The observation that motor neurons' death can be prevented after lesion in the early postnatal period could introduce novel therapeutic approaches to the treatment of motor neuron degeneration. For example, it will be of interest to investigate whether CNTF also protects against toxic or genetic degenerative changes in motor neurons, and possibly also in those motor neuron diseases of unknown aetiology. □

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1. Sheard, P., McCaig, C. D. & Harris, A. J. *Devl Biol.* **102,** 21–31 (1984).
2. Schmalbruch, H. *J. comp. Neurol.* **224,** 252–258 (1984).
3. Gudden, B. *Arch. Psychiat. Nervenkrankh.* **2,** 693–723 (1870).
4. Stöckli, K. A. *et al. Nature* **342,** 920–923 (1989).
5. Lin, L.-F. H. *et al. Science* **246,** 1023–1025 (1989).
6. Barbin, G., Manthorpe, M. & Varon, S. *J. Neurochem.* **43,** 1468–1478 (1984).
7. Kreutzberg, G. W. in *The Facial Nerve* (ed. May, M.) 75–83 (Thieme, New York, 1986).
8. Nissl, F. *Allg. Z. Psychiat.* **48,** 197–198 (1892).
9. Graeber, M. B. & Kreutzberg, G. W. *J. Neurocytol.* **15,** 363–373 (1986).
10. Tetzlaff, W., Graeber, M., Bisby, M. A. & Kreutzberg, G. W. *Glia* **1,** 90–95 (1988).
11. Bignami, A. & Dahl, D. *Nature* **252,** 55–56 (1974).
12. Springall, D. R., Hacker, G. W., Grimelius, L. & Polak, J. M. *Histochemistry* **81,** 603–608 (1984).
13. Ernsberger, U., Sendtner, M. & Rohrer, H. *Neuron* **2,** 1275–1284 (1989).
14. Oppenheim, R. W. *J. comp. Neurol.* **246,** 281–286 (1986).

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The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene

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MICE homozygous for the recessive mutation osteopetrosis (*op*) on chromosome 3 have a restricted capacity for bone remodelling, and are severely deficient in mature macrophages and osteoclasts[1–3]. Both cell populations originate from a common haemopoietic progenitor. As *op/op* mice are not cured by transplants of normal bone marrow cells[4], the defects in *op/op* mice may be associated with an abnormal haematopoietic microenvironment rather than with an intrinsic defect in haematopoietic progenitors. To investigate the molecular and biochemical basis of the defects caused by the *op* mutation, we established primary fibroblast cell lines from *op/op* mice and tested the ability of these cell lines to support the proliferation of macrophage progenitors. We show that *op/op* fibroblasts are defective in production of functional macrophage colony-stimulating factor (M-CSF), although its messenger RNA (*Csfm* mRNA) is present at normal levels. This defect in M-CSF production and the recent mapping of the *Csfm* structural gene near *op* on chromosome 3 (refs 5, 6) suggest that *op* is a mutation within the *Csfm* gene itself. We have sequenced *Csfm* complementary DNA prepared from *op/op* fibroblasts and found a single base pair insertion in the coding region of the *Csfm* gene that generates a stop codon 21 base pairs downstream. Thus, the *op* mutation is within the *Csfm* coding region and we conclude that the pathological changes in this mutant result from the absence of M-CSF.

Previous reports show that macrophage numbers are markedly reduced in various tissues from *op/op* mice[2]. In order to investigate the molecular basis of the macrophage defect in *op/op* mice *in vitro*, lung fibroblast lines were established from B6C3-*op/op* and +/? normal littermate mice. These fibroblast lines were tested for their ability to produce factors that support the proliferation of normal macrophage precursors. BALB/c bone marrow cells (passed twice through Sephadex G-10 columns) were co-cultured with the fibroblast lines for two weeks. Massive proliferation of haemopoietic cells occurred with fibroblasts from normal littermate mice (Fig. 1*a*), and the majority of recovered cells ($98.5 \pm 1.2\%$) were macrophages (Fig. 1*b*). In contrast, proliferation of bone marrow cells was severely reduced with fibroblasts from *op/op* mice and the majority of recovered cells were granulocytes, although a small percentage of macrophages were also detected ($5.8 \pm 0.9\%$) (Fig. 1*c*). This result shows that the macrophage microenvironment defect of *op/op* mice is reproduced *in vitro* using the lung fibroblast culture system.

To determine whether the failure of *op/op* fibroblast cultures to support normal macrophage development is due to a defect in the production of growth factor(s) which induce the proliferation of macrophage precursors, colony-stimulating activity in the culture supernatant of *op/op* fibroblasts was assessed. It induced a fifth of the number of colonies induced by culture supernatant from normal fibroblasts. Moreover, anti-GM-CSF

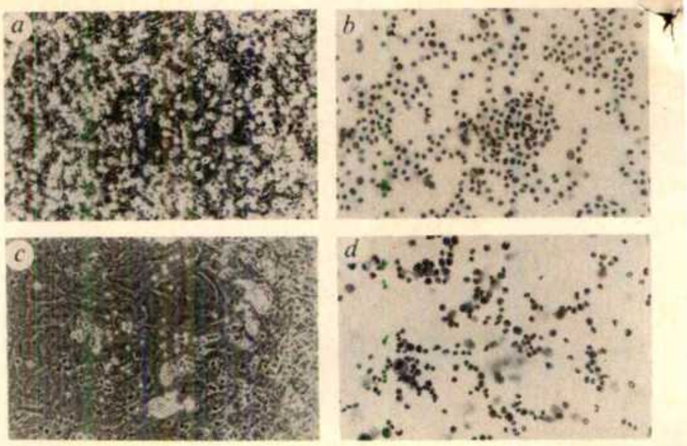

FIG. 1 Low efficiency of *op/op* fibroblasts in supporting the proliferation of macrophage precursors. BALB/c bone marrow cells (depleted of stromal cells by passage through Sephadex G-10 columns) were cultured either on *op/op* or +/?-littermate fibroblasts for 2 weeks. *a*, Phase contrast view of the culture on littermate fibroblasts and *c*, on *op/op* fibroblasts. May-Grunwald-Giemsa staining of the cells present in the culture of *b*, littermate fibroblasts and *d*, *op/op* fibroblasts.

METHODS. Preparation of primary fibroblast cell lines: Lung tissues were minced and incubated with 500 U ml^{-1} collagenase (Sigma) in RPMI 1640 medium (GIBCO) containing 5% calf serum (Hyclone), 5×10^{-5} M 2-mercaptoethanol (Sigma), 50 µg ml^{-1} streptomycin and 50 U ml^{-1} penicillin (Meiji Seika, Tokyo) in 5% CO_2 at 37 °C for 12 h. After washing three times with the same medium, the total lung cells from individual mice were seeded in 100-mm tissue culture dishes (Corning). The cultured cells were grown to confluency, collected by trypsin-EDTA treatment, and subcultured into six dishes. This protocol was repeated over 10 times, and the cells obtained were used as fibroblast cell lines in this study. We prepared fibroblast lines from three pairs of *op/op* and littermate +/? mice at 3-, 4-, and 6-weeks of age. Preparation of bone marrow cells and co-culture with fibroblasts: To remove mature macrophages and adherent stromal cells, BALB/c bone marrow cells were passed through a Sephadex G-10 column twice (100×10^6 cells per 20 ml, Pharmacia) and 10^6 of the eluted cells were seeded on confluent fibroblast layers in T25 flasks (Corning). The medium (5% calf serum, 5×10^{-5} 2-mercaptoethanol, RPMI 1640 (6 ml)) was changed every three days.

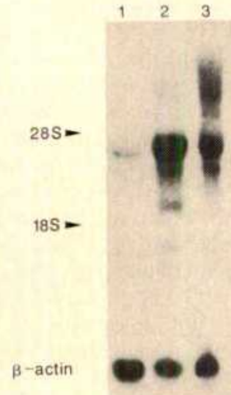

FIG. 2 Expression of M-CSF transcripts in *op/op* fibroblasts. Total RNA was prepared from *op/op* (lane 3), +/?-littermate (lane 2), fibroblasts or EL4 thymoma cell lines (lane 1). 20 µg of each RNA was separated by PAGE and blotted onto nitrocellulose membrane (Hybond-C; NEN). A full-length *Csfm* cDNA clone (CDM8MCSFCL7) was used as a hybridization probe. The murine β-actin probe used as a control indicates equal loading.

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antiserum abolished all the colony-stimulating activity of *op/op* fibroblasts. Thus, GM-CSF was the only detectable colony-stimulating activity secreted by the *op/op* fibroblasts (Table 1). In contast, 80% of colonies induced by the culture supernatant of normal littermate fibroblasts remained after anti-GM-CSF antiserum treatment. The macrophage-inducing factors expressed in normal fibroblasts are GM-CSF and M-CSF (refs 7–9). Our results show that M-CSF production is defective in *op/op* mice.

To test whether *op/op* fibroblasts are defective in *Csfm* mRNA production, mRNA was prepared from *op/op* or littermate fibroblasts and expression of the *Csfm* gene measured by northern blot analysis. Despite the absence of functional M-CSF activity in *op/op* fibroblasts, the level of *Csfm* mRNA expression was normal, indicating that it is the protein that is aberrant in *op/op* mice (Fig. 2).

To characterize the mutation in the *Csfm* gene of *op/op* mice we prepared cDNA from *op/op* fibroblast lines and amplified the gene using the polymerase chain reaction[10] (PCR), before cloning into Bluescript (pBS) M13$^+$ vectors. As M-CSF activity is present in the N-terminal third of the *Csfm* gene[11-14] only cDNA corresponding to this region was sequenced. Four independent clones had a T (thymidine) insertion 262 base pairs (bp) downstream from the ATG initiation codon by comparison with *Csfm* cDNA cloned from mouse L-cells[12]. No such insertion was detected in the *Csfm* cDNA from a normal stromal cell line, ST2, which secretes functional M-CSF (refs 15, 16; Fig. 3). Because this insertion generates a TGA stop codon 21 bp further downstream we conclude that the *Csfm* gene of *op/op* mice cannot code for functional M-CSF protein. Although PCR amplification can generate random errors, it is unlikely to cause errors at the same position in four independent clones. The *Csfm* gene has recently been mapped and found to be close to the *op* locus on chromosome 3 (refs 5, 6), supporting our conclusion that the *op* locus is the *Csfm* gene itself.

In conclusion, the phenotypic abnormality in *op/op* mice is caused by the lack of a specific cytokine acting on haemopoietic cells. Such mutants, although severely defective in bone remodelling, are relatively healthy when maintained under pathogen-free conditions, suggesting that production of M-CSF is not necessary for the maintenance of various types of M-CSF responsive cells other than osteoclasts. The fact that *op/op* mice produced from matings of +/*op* heterozygotes appear normal at birth raises a question as to whether bone remodelling during early development is independent of M-CSF. This question is difficult to answer, because M-CSF is produced by maternal uterine epithelial cells directly above the decidual layer of placenta and is expected to be present in the tissues of *op/op* embryos[17,18]. Thus, bone remodelling of the *op/op* fetus may proceed normally using maternal M-CSF, resulting in the normal development of the embryo. Further investigation of the pathophysiology of *op/op* mice, in the light of our present findings, will provide an insight into the role of M-CSF *in vivo* during postnatal life. □

TABLE 1 Absence of CSF-production other than GM-CSF from *op/op* fibroblasts

		No. of colonies per 10^5 bone marrow cells			
Factors		Medium	NRS	Anti-IL-3	Anti-GM-CSF
Medium		0	0	0	0
IL-3	(100 U)	199±1	213±52	0	213±1
GM-CSF	(100 U)	149±15	146±24	170±15	0
LCCM	(10%)	112±12	89±3	98±5	76±20
+/? SN	(10%)	210±13	214±12	211±25	174±19
	(2%)	164±26	164±10	160±18	131±21
op/op SN	(10%)	44±12	42±8	41±1	3±3
	(2%)	3±1	1±1	2±1	0

Colony formation assays in soft agar (DIFCO) were carried out as described[19]. Positive controls were: recombinant mouse IL-3, GM-CSF and L-cell conditioned medium (LCCM) as a source of M-CSF. Tissue culture supernatant was prepared from primary lung-fibroblast cell lines derived from +/?-littermates or *op/op* mice by incubating confluent fibroblast cultures for five days in fresh medium. To identify the factors secreted into the culture supernatant, we used rabbit antisera against murine GM-CSF or IL-3. We used 1% dilutions of the antibodies, which can saturate ~4 U ml^{-1} for IL-3 and 20 U ml^{-1} for GM-CSF. An equivalent amount of non-immune normal rabbit serum (NRS) was used as a control. Each value represents arithmetic mean ± standard deviation for triplicate cultures.

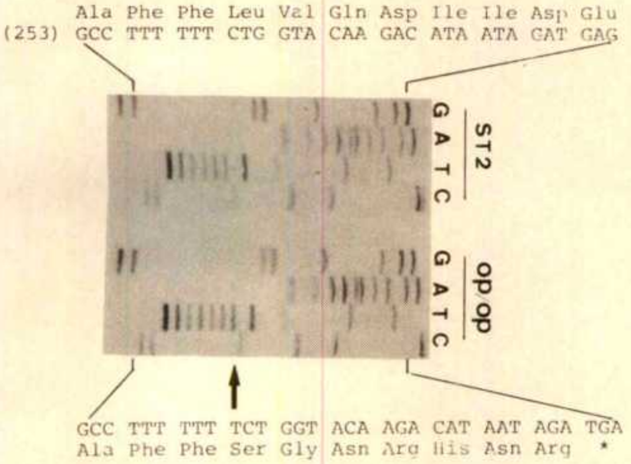
Ala Phe Phe Leu Val Gln Asp Ile Ile Asp Glu
(253) GCC TTT TTT CTG GTA CAA GAC ATA ATA GAT GAG
G
A
T
C
ST2
G
A
T
C
op op
GCC TTT TTT TCT GGT ACA AGA CAT AAT AGA TGA
Ala Phe Phe Ser Gly Asn Arg His Asn Arg *

FIG. 3 One-base pair insertion in the coding region of the *Csfm* gene of *op/op* mutant. The coding region of the *Csfm* gene expressed in *op/op* fibroblasts was cloned using PCR and the nucleotide sequence was determined. Nucleotide sequence of *Csfm* cDNA clone at the site of mutation; *op/op* (clone pBSop-1) and ST2 (pBSST2). The same insertion was detected in three additional independent clones from *op/op* fibroblasts, whereas no such insertion was detected in four independent clones from normal fibroblasts.

METHODS. cDNA was prepared from 10 μg total RNA using a cDNA synthesis kit (BRL) with oligo(dT) primer and used as the template for PCR amplification of the *Csfm* coding region[10]. Oligonucleotide primers for PCR amplification were CCGGGATCCAGCTGCCCGT (5′ primer) and ATAGAATTCTTTCTATACTGGCAGTTC (3′ primer), synthesized by an automated DNA synthesizer (ABI). The conditions used were; 2.5 U *Taq* DNA polymerase (BRL), 100 pmol of primers in 50 μl 2 mM $MgCl_2$, 50 mM KCl, 25 mM TAPS buffer (pH 9.3), 1 mM DTT and 30 cycles in a Program Temp Control System PC-500 (ASTEC, Kitakyushu, Japan). Amplified genes were digested with *Hin*dIII and *Eco*RI restriction enzymes (NEB), purified by agarose gel electrophoresis, cloned into pBSM13$^+$ (Stratagene) and sequenced. Primers used for sequence reactions were GAACTCTCCAATAACCTG, GAACAGCTGGATGATCCTG and CAGGATCATCCAGCTGTTC (which are the sequences in the coding region of *Csfm* gene) and T3 and T7 primers (Stratagene).

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1. Marks Jr. S. C. & Lane, P. W. *J. Heredity* **67**, 11–18 (1976).
2. Wiktor-Jedrzejczak, W., Ahmed, A., Szczylik, C. & Skelly, R. R. *J. exp. Med.* **156**, 1516–1527 (1982).
3. Shultz, L. D. & Sidman, C. *A. Rev. Immun.* **5**, 367–403 (1987).
4. Marks Jr. S. C., Seifert, M. F. & McGuire, J. L. *Metab. Bone Dis. & Rel. Res.* **5**, 183–186 (1984).
5. Buchberg, A. M., Jenkins, A. A. & Copeland, N. G. *Genomics* **5**, 363–367 (1989).
6. Gisselbrecht, S. *et al. Blood* **73**, 1742–1746 (1989).
7. Sachs, L. *Science* **238**, 1374–1379 (1987).
8. Metcalf, D. *Nature* **339**, 27–30 (1989).
9. Stanley, E. R. *et al. J. cell. Biochem.* **21**, 151–159 (1983).
10. Saiki, R. K. *et al. Science* **239**, 487–491 (1988).
11. Rajavashisth, T. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 1157–1161 (1987).
12. Ladner, M. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 6706–6710 (1988).
13. Ladner, M. B. *et al. EMBO J.* **6**, 2693–2698 (1987).
14. Takahashi, M. *et al. Biochem. biophys. Res. Commun.* **161**, 892–901 (1989).
15. Ogawa, M. *et al. EMBO J.* **7**, 1337–1343 (1988).
16. Nishikawa, S. I., Ogawa, M., Nishikawa, S., Kunisada, T. & Kodama, H. *Eur. J. Immun.* **18**, 1767–1771 (1988).
17. Regenstreif, L. J. & Rossant, *J. dev Biol.* **133**, 284–294 (1989).

18. Polland, J. W. *et al. Nature* **330,** 484-486 (1987).
19. Kincade, P. W., Ralph, P. & Moore, M. A. S. *J. exp. Med.* **143,** 1265-1270 (1976).

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Disruption of a C/EBP binding site in the factor IX promoter is associated with haemophilia B

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HAEMOPHILIA B (or Christmas disease) is an inherited, X-linked bleeding disorder caused by mutations in the gene for clotting factor IX. There is a rare class of patients, exemplified by haemophilia B Leyden[1], who suffer from haemophilia B as children but improve after puberty. In these patients, plasma factor IX concentrations are less than 10% of normal during childhood, but after puberty they gradually rise to between 40 and 80% of normal. Mutations clustered around the main transcription start point (defined as +1 (ref. 2)) have been reported in seven of these patients (at −20 (refs 1, 3, 4); −6 (refs 5, 6) and +13 (refs 7, 8)). To determine how these mutations interfere with factor IX expression, we have assayed for transcription factors binding to this area and have identified a nuclear factor-1 liver (NF1-L) binding site[9] (−99 to −76) and a binding site for the CCAAT/enhancer binding protein (C/EBP)[10] (+1 to +18). We show that the A → G mutation at +13 prevents the binding of C/EBP to this site. Furthermore, we show that C/EBP is capable of transactivating a cotransfected normal factor IX promoter but not the mutant promoter. This is the first natural mutation to be reported which disrupts a C/EBP binding site and is an illustration of the importance of this transcription factor in humans.

Factor IX, like the other vitamin K-dependent serine proteases involved in blood clotting, is expressed primarily in the liver[11]. Therefore we used DNase I footprinting with rat liver nuclear extracts to analyse the region implicated by the point mutations. Two footprints were observed (Fig. 1). The factor binding to the distal element (−99 to −76) was heat-sensitive, the other (+1 to +18) was heat-stable (Fig. 1*a*). The distal footprint was observed using HeLa, human hepatoma cell lines (HepG2 and Hep3B (ref. 12)) and rat liver extracts, the proximal footprint only in rat liver extracts (Fig. 1 and our unpublished results). These observations together with a consideration of known consensus binding sites[13-15], led us to suspect that the distal footprint was caused by one of the nuclear factor-1 family of proteins and the proximal by C/EBP.

To test whether these proteins could indeed bind to these regions, we used an NF1-L fragment (containing N-terminal residues 4-240 and full DNA-binding activity[9]; gift from R. Cortese) and a C/EBP fragment (containing the 88 C-terminal residues and also capable of binding DNA[16]; gift from S. McKnight) in our binding assays. We show that these proteins do indeed bind to the expected sites and produce footprints essentially identical to those produced by nuclear extracts (Figs 1*b* and 2*a*).

As the A→G mutation at +13 (found in two unrelated individuals with Leyden-like haemophilia B[7,8]) lies within the C/EBP binding site, we tested whether this mutation affected C/EBP binding. In our assays it abolished binding (Fig. 2*b* and *c*). This result was confirmed in competition experiments, where it was found that an oligonucleotide (residues −27 to +30) carrying the +13 mutation competed out the footprint roughly 100 times less efficiently than an equivalent normal oligonucleotide (data not shown).

The G→A mutation at −6 (also associated with the Leyden condition; ref. 5 and our unpublished results), is adjacent to the region protected by C/EBP (+1 to +18). Therefore it could affect C/EBP binding, either directly or indirectly, by generating a site for a second, unrelated DNA-binding protein which might itself prevent the binding of C/EBP. To investigate this possibility, a DNA fragment containing residues −189 to +21 and carrying the −6 mutation was used in footprinting experiments with rat liver extract. The −6 G→A mutation did not affect the proximal (C/EBP, +1 to +18) footprint or the distal (NF1-L, −99 to −76) footprint, nor was any new footprint seen (data not shown). This result indicates that the −6 mutation works by a different mechanism from that of the +13 mutation. Further evidence that this is the case is presented below.

Functional studies of the factor IX promoter are difficult, for several reasons. First, unlike most genes studied to date the

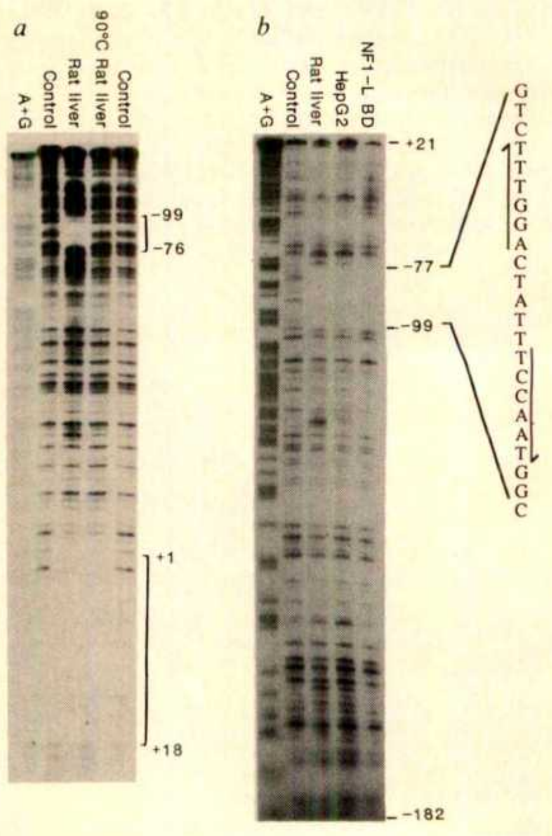

FIG. 1 DNase I footprinting of the factor IX promoter region. *a*, Restriction fragment (containing residues −189 to +21), ^{32}P end-labelled on the sense-strand and footprinted with either rat liver nuclear extract (20 μg), or a similar extract heated at 90 °C (10 min). Control lanes, throughout, contain no protein. A+G throughout refers to Maxam-Gilbert sequencing using formic acid. *b*, Same restriction fragment, end-labelled on the anti-sense strand and footprinted with rat liver nuclear extract (20 μg), HepG2 extract (20 μg) or purified NF1-L BD (NF1-L DNA-binding domain[9] (60 ng)). The −189 to +21 fragment is derived from a *Hinf*I to *Nhe*I fragment, filled-in with Klenow and cloned into the *Sma*I site of pCAT00 (see Fig. 3 legend). To label the sense-strand, this fragment was cut with *Sau*3A, end-labelled using Klenow, excised by recutting with *Asp*718 and purified on a 6% non-denaturing polyacrylamide gel. The anti-sense strand was labelled similarly but was first cut with *Asp*718. Rat liver and HepG2 nuclear extracts were prepared by standard methods[24-26]. End-labelled fragment (1-2 ng) was used in footprinting reactions (40 μl total volume) in 10 mM HEPES buffer, pH 7.8, 40 mM KCl, 3 mM $MgCl_2$, 4% Ficoll, 0.5 mM DTT and 2 μg poly(dI·dC). Proteins or extract were added first then, after 10 min at 20 °C, probe was added and after a further 10 min the mixture was digested (30-120 s) with 1 U RQ1 DNase (Promega). Samples were extracted with phenol-chloroform, precipitated with ethanol and analysed on 8% polyacrylamide-7M urea gels.

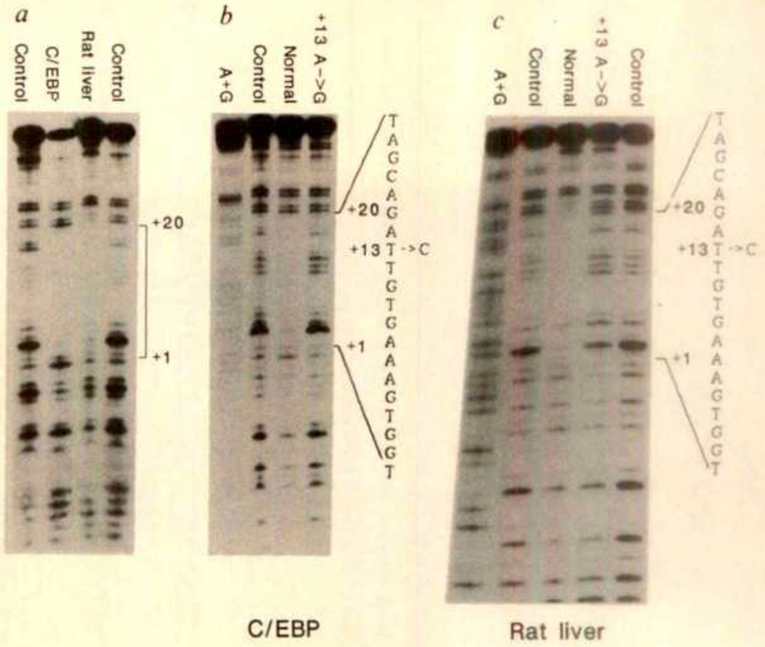

FIG. 2 DNase I footprinting of the proximal region, comparing normal and mutant probes. *a*, Footprinting of a restriction fragment (containing residues −27 to +30 and end-labelled on the anti-sense strand) with either purified C/EBP fragment (10 ng)[16] or rat liver nuclear extract (20 μg). Controls contain no added protein. *b*, *c*, Footprinting of the same fragment and a similar fragment containing the +13 A → G mutation, with C/EBP fragment (10 ng) (*b*) or rat liver extract (20 μg) (*c*). The normal and mutant −27 to +30 fragments were obtained by cloning synthetic oligonucleotides into the *Sma*I site of pCATOO. End-labelling and footprinting reactions were as in Fig. 1 except that, where C/EBP was used, only 50 ng poly(dI·dC) was added to the footprinting mix. A + G, See legend to Fig. 1.

factor IX gene is expressed at low levels (the plasma concentration of factor IX is 5 μg ml^{-1} compared with that of albumin, 20–50 mg ml^{-1}), consequently only very sensitive promoter assays are informative. Second, there is only one continuous cell line known to produce endogenous factor IX, namely HepG2. These cells do not produce detectable levels of secreted factor IX (ref. 12 and our unpublished results) but do produce small amounts of intracellular protein and messenger RNA[17].

We linked a region presumed to contain the factor IX promoter (−2,258 to +21) to the reporter gene chloramphenicol acetyl transferase (CAT) and assayed for CAT activity in transiently-transfected HepG2, Hep3B and HeLa cells. We used a new, low-background vector (plasmid pCATOO), which contains two synthetic polyadenylation sites[18] upstream of the *CAT* gene (Fig. 3). The region −2,258 to +21 is sufficient to produce low but detectable levels of CAT enzymatic activity in HepG2 cells. Only background levels of activity were observed in Hep3B and HeLa (unpublished results). A series of deletion constructs were generated using *Bal*31 or convenient restriction sites and constructs were tested for activity in HepG2 cells (Fig. 3*a*). Deletions from −2,258 to −98 had no significant effect, but a further deletion to −77 caused a fivefold reduction in CAT activity. This result supports the hypothesis that the NF1-L binding site (−99 to −76) is important *in vivo*.

The −6 G → A and +13 A → G mutations were also tested in this functional assay. The −6 mutation reduced CAT activity to background levels but, surprisingly, the +13 mutation did not impair promoter activity (Fig. 3*b*). To test whether this was a consequence of the low concentrations of C/EBP in HepG2 cells[19], we repeated the experiment in HepG2 cells simultaneously transfected with an expression plasmid for C/EBP (gift from S. McKnight[19]). C/EBP was capable of transactivating the normal promoter but it could not activate above background the promoter carrying the +13 A → G mutation (Fig. 3*c*). The background transactivation of pCATOO is most probably due to a cryptic C/EBP binding site(s) within the vector. Although

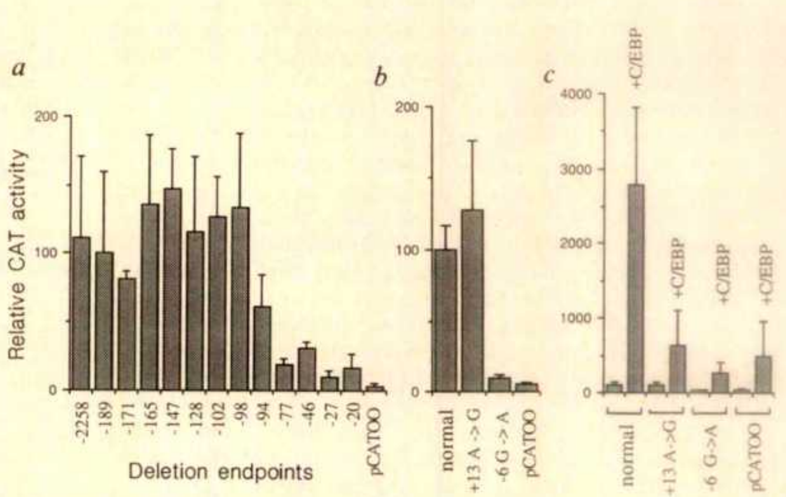

FIG. 3 Summary of the CAT assays in HepG2 cells. *a*, Relative activities of *Bal*31 deletion constructs. *b*, Comparison of normal promoter sequences (−189 to +21) with those carrying the +13 A → G and −6 G → A mutations. *c*, Comparison of the extents to which normal and mutant promoters are transactivated by C/EBP. The amount of CAT activity throughout is shown relative to plasmid *pf*IX-189 (see below), which is arbitrarily set at 100. The columns labelled 'normal' in (*b*) and (*c*) refer to this plasmid. All results are compiled from at least three independent experiments carried out with at least two independent preparations of plasmid. Standard errors around the mean are shown. The low-background CAT plasmid, pCATOO, was a modification of the previously described pUC119-based plasmid, pCATO (ref. 27; gift from P. Lamb). Plasmid pCATO was cut with *Sst*II, the site was blunt-ended and a *Bgl*II linker added. Two synthetic polyA sites[18] (gifts from N. Proudfoot) were cloned into this site to form pCATOO. The promoter constructs were prepared as follows: pfIX-2,258 was made by cloning a blunt-ended *Nhe*I fragment (−2,258 to +21) into the *Sma*I site of pCATOO, which lies immediately upstream of the CAT gene; pfIX-189 was similarly constructed using a blunt-ended −189 to +21 *Hinf*I to *Nhe*I fragment. The remaining constructs were generated by *Bal*31-mediated deletion. HepG2 cells were grown on MEM medium supplemented with non-essential amino-acids and 10% FCS. They were transfected[28] when 10–20% confluent. The β-galactosidase-encoding plasmids, pIRV[29] or pCH110[30] were used as cotransfection controls. Extracts were prepared after 48 h and CAT and β-galactosidase activities measured[30]. In *a* and *b*, CAT plasmid (30 μg) and pIRV (2 μg) were used. The transactivation experiment (*c*) was performed as described[19]. CAT plasmid (5 μg) and pCH110 (2 μg) were transfected together with either sonicated herring testes DNA (15 μg) or the C/EBP expression plasmid pMSV/C/EBP.

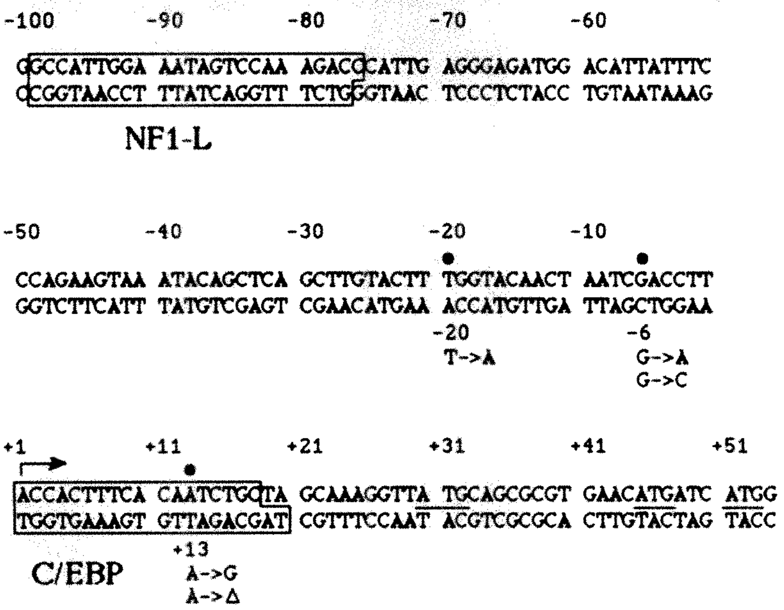
-100 -90 -80 -70 -60
GGCCATTGGA AATAGTCCAA AGACCCATTG AGGGAGATGG ACATTATTTC
CCGGTAACCT TTATCAGGTT TCTGGGTAAC TCCCTCTACC TGTAATAAAG
NF1-L
-50 -40 -30 -20 -10
CCAGAAGTAA ATACAGCTCA GCTTGTACTT TGGTACAACT AATCGACCTT
GGTCTTCATT TATGTCGAGT CGAACATGAA ACCATGTTGA TTAGCTGGAA
-20
T->A
-6
G->A
G->C
+1 +11 +21 +31 +41 +51
ACCACTTTCA CAATCTGCTA GCAAAGGTTA TGCAGCGCGT GAACATGATC ATGG
TGGTGAAAGT GTTAGACGAT CGTTTCCAAT ACGTCGCGCA CTTGTACTAG TACC
+13
A->G
A->Δ
C/EBP

FIG. 4 Sequence of the 5′ flanking region of the factor IX gene, containing the minimal promoter as defined by CAT assays in HepG2 cells. NF1-L and C/EBP binding sites are boxed. The primary start point of transcription[2] is marked with an arrow and potential translation-initiation codons are underlined. Previously described mutations known to be associated with the Leyden form of haemophilia B are indicated by dots (−20 T→A (refs 1, 3, 4); −6 G→A (ref. 5) and our unpublished results); −6 G→C (ref. 6); +13 A→G (refs 7, 8); and the single base deletion (Δ) at +13 (ref. 7).

such transactivation experiments may not reflect the *in vivo* situation in quantitative terms, these results clearly demonstrate that C/EBP can activate the factor IX promoter and that the +13 mutation interferes with this activation. Furthermore, the fact that the +13 mutation impairs promoter function only when tested in HepG2 cells transfected with a C/EBP encoding plasmid, whereas the −6 mutation reduces promoter activity in both systems (Fig. 3*b* and *c*), supports our assertion that the −6 and +13 mutations exert their effects through different mechanisms.

In conclusion, we have identified an NF1-L (−99 to −76) and a C/EBP binding site (+1 to +18) in the 5′ flanking region of the human factor IX gene (Fig. 4). The results obtained from *in vivo* assays of promoter activity, carried out in HepG2 cells, are consistent with the *in vitro* footprinting results. Deletion of the NF1-L binding site caused a fivefold reduction in CAT activity. The +13 A→G mutation, which is associated with haemophilia B[7,8] and disrupts a C/EBP binding site *in vitro*, did not impair promoter activity in ordinary HepG2 cells but did in HepG2 cells cotransfected with a C/EBP expression plasmid. The −6 G→A mutation associated with haemophilia B caused a significant reduction in CAT activity in our assays. The mutation did not influence the binding of NF1-L or C/EBP in footprinting experiments. It is probable that as-yet-uncharacterized protein(s) bind to the −6/−20 region(s).

Although we have shown that there are NF1-L and C/EBP binding sites within the minimal promoter, as defined in HepG2 cells, and that C/EBP can transactivate the promoter, it is possible that other proteins may also bind to these sites and be important for transcription *in vivo*. Other members of the NF1 family[20] may bind. Moreover it has recently been shown that C/EBP is one of a family of proteins, encoded by separate genes (Z. Cao and S. McKnight; P. Johnson, personal communication). These proteins possess closely related DNA-binding domains and may also be capable of interacting with the factor IX promoter.

Although C/EBP binding sites have been found both upstream and downstream of many transcription start sites[15], the finding of a binding site so close to the start site is unusual. The significance of this finding, in relation to the role of C/EBP during the initiation and process of transcription remains to be defined.

The significant increase in factor IX expression in haemophilia B Leyden patients after puberty is not understood, although it is directly or indirectly due to increasing levels of testosterone[4,21]. We feel it is best explained by postulating the existence of a nearby androgen-responsive element, which contributes to promoter strength in these patients after puberty[7]. We are aware of no evidence that the promoter is responsive to androgen in the normal population.

Naturally occurring promoter mutations are a rare and promising source of information on mammalian gene expression and transcription factors[22]. C/EBP in particular may be important in fully differentiated, non-proliferating cells[19,23]. The finding that the disruption of a C/EBP binding site is associated with haemophilia B in humans supports the view that C/EBP (or, more correctly, the human analogue of the rat protein C/EBP) plays an important part in gene expression in the liver *in vivo*. ☐

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1. Veltkamp, J. J., Meilof, J., Remmelts, H. G., Van der Vlerk, D. & Loeliger, E. A. *Scand. J. Haemat.* **7**, 82–90 (1970).
2. Anson, D. S. *et al. EMBO J.* **3**, 1053–1060 (1984).
3. Briët, E., Bertina, R. M., Van Tilburg, N. H. & Veltkamp, J. J. *New Engl. J. Med.* **306**, 788–790 (1982).
4. Reitsma, P. H., Bertina, R. M., Ploos van Amstel, J. K., Riemens, A. & Briët, E. *Blood* **72**, 1074–1076 (1988).
5. Kurachi, K., Hirosawa, S., Fahner, J. B., Wu, C. T. & Salier, J. P. *Thromb. Haemostasis* **62**, 154 (1989).
6. Attree, O., Vidaud, M., Vidaud, D., Lavergne, J. M. & Goosens, M. *Thromb. Haemostasis* **62**, 8 (1989).
7. Reitsma, P. H., Mandalaki, T., Kasper, C. K., Bertina, R. M. & Briët, E. *Blood* **73**, 743–746 (1989).
8. Crossley, P. M., Winship, P. R., Black, A., Rizza, C. R. & Brownlee, G. G. *Lancet* **i**, 960 (1989).
9. Paonessa, G., Gounari, F., Frank, R. & Cortese, R. *EMBO J.* **7**, 3115–3123 (1988).
10. Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J. & McKnight, S. L. *Genes Dev.* **2**, 786–800 (1988).
11. Fair, D. S. & Bahnak, B. R. *Blood* **64**, 194–204 (1984).
12. Aden, D. P., Fogel, A., Plotkin, S., Damjanov, I. & Knowles, B. B. *Nature* **282**, 615–616 (1979).
13. Nowock, J., Borgmeyer, U., Püschel, A. W., Rupp, R. A. W. & Sippel, A. E. *Nucleic Acids Res.* **13**, 2045–2061 (1985).
14. Gronostajski, R. M. *Nucleic Acids Res.* **15**, 5545–5559 (1987).
15. Ryden, T. A. & Beemon, K. *Molec. cell. Biol.* **9**, 1155–1164 (1989).
16. Vinson, C. R., Sigler, P. B. & McKnight, S. L. *Science* **246**, 911–916 (1989).
17. Huang, M-N., Smith, K., Evans, J. P., Wolde, S. T. & High, K. A. *Thromb. Haemostasis* **62**, 81 (1989).
18. Levitt, N., Briggs, D., Gil, A. & Proudfoot, N. J. *Genes Dev.* **3**, 1019–1025 (1989).
19. Friedman, A. D., Landschulz, W. H. & McKnight, S. L. *Genes Dev.* **3**, 1314–1322 (1989).
20. Santoro, C., Mermod, N., Andrews, P. C. & Tjian, R. *Nature* **324**, 218–224 (1988).
21. Briët, E., Wijnands, M. C. & Veltkamp, J. J. *Ann. Intern. Med.* **103**, 225 (1985).
22. Martin, D. I. K., Tsai, S-F. & Orkin, S. H. *Nature* **338**, 435–438 (1989).
23. Birkenmeier, E. H. *et al. Genes Dev.* **3**, 1146–1156 (1989).
24. Graves, B. J., Johnson, P. F. & McKnight, S. L. *Cell* **44**, 565–576 (1986).
25. Gorski, K., Carneiro, M. & Schibler, U. *Cell* **47**, 767–776 (1986).
26. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. *Nucleic Acids Res.* **11**, 1475–1489 (1983).
27. Lamb, P., Watt, P. & Proudfoot, N. J. *Progress in Clinical and Biological Research, Haemoglobin Switching* Vol. **316**, 269–278 (Liss, New York, 1989).
28. Graham, F. L. & van der Eb, A. J. *Virology* **52**, 456–467 (1973).
29. Beddington, R. S. P., Morgernstern, J., Land, H. & Hogan, A. *Development* **106**, 37–46 (1989).
30. Herbomel, P., Bourachot, B. & Yaniv, M. *Cell* **39**, 653–662 (1984).

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Morphological transformation of human keratinocytes expressing the LMP gene of Epstein–Barr virus

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THE association of Epstein-Barr virus (EBV) with nasopharyngeal carcinoma (NPC) has been known for some time[1,2], but the precise role of EBV in this cancer is poorly understood, due partly to the lack of an *in vitro* system for studying NPC cells and the effect of EBV on epithelial cells. Biopsies of NPC tumours have revealed expression of the EBV latent membrane protein (LMP) in 65% of cases[3], suggesting that in at least some NPC tumours LMP may contribute to cell transformation. Here we address the question of the effect of LMP expression on epithelial cells. Transfection of an immortalized, non-tumorigenic keratinocyte cell line (RHEK-1) with the LMP gene causes a striking morphological transformation: the originally flat, polygonal colonies change to bundles of spindle-shaped cells that form multilayer foci, and cytokeratin expression is down-regulated. Our results suggest that LMP expression may be an important causal factor in the development of NPC.

RHEK-1 cells were transfected with either plasmid pJ132 or pJ144, which carry the genes for LMP and EBNA-2 (EBV nuclear antigen) respectively and the guanine phosphoribosyl transferase selectable marker. After selection on medium containing mycophenolic acid and xanthine, cells were analysed for the expression of EBNA-2 and LMP respectively (Fig. 1). A band of relative molecular mass (M_r) 90,000 (90K), corresponding to the expected EBNA-2 size, was detected in the 100% positive EBNA-2-transfected clones, but not in the LMP-transfected or the control cells. Six different LMP-transfected clones all contained a new 63K band, detected by the anti-LMP monoclonal antibody, S-12. This band corresponded in size, to the known LMP band of EBV-transformed lymphoblastoid cell line, IB-4, and was not detected in the EBNA-2 transfectant or in the EBV-negative control line.

PKK-1 and PKK-2 are monoclonal antibodies against cytokeratins. PKK-1 reacts against cytokeratins 19, 18, 8 and PKK-2 against 19, 17, 16 and 7 (ref. 4). AE1/AE3 is a pooled mixture of antibodies that reacts against cytokeratin antigens of M_r values between 40 and 67K (ref. 5). PKK-1 and PKK-2 reacted against a band of approximately 40K in the control and in EBNA-2-transfected RHEK-1 cells. In addition two weak bands at approximately 90–95K not corresponding to any known cytokeratins were detected with the PKK-2 antibody in RHEK-1 and in cells transfected with EBNA-2. Neither of these bands appeared in the LMP-transfected cells (Fig. 2*a*, *b*). The AE1/AE3 mixture reacted against a variety of bands between approximately 40 and 66K in the control cells. A similar but

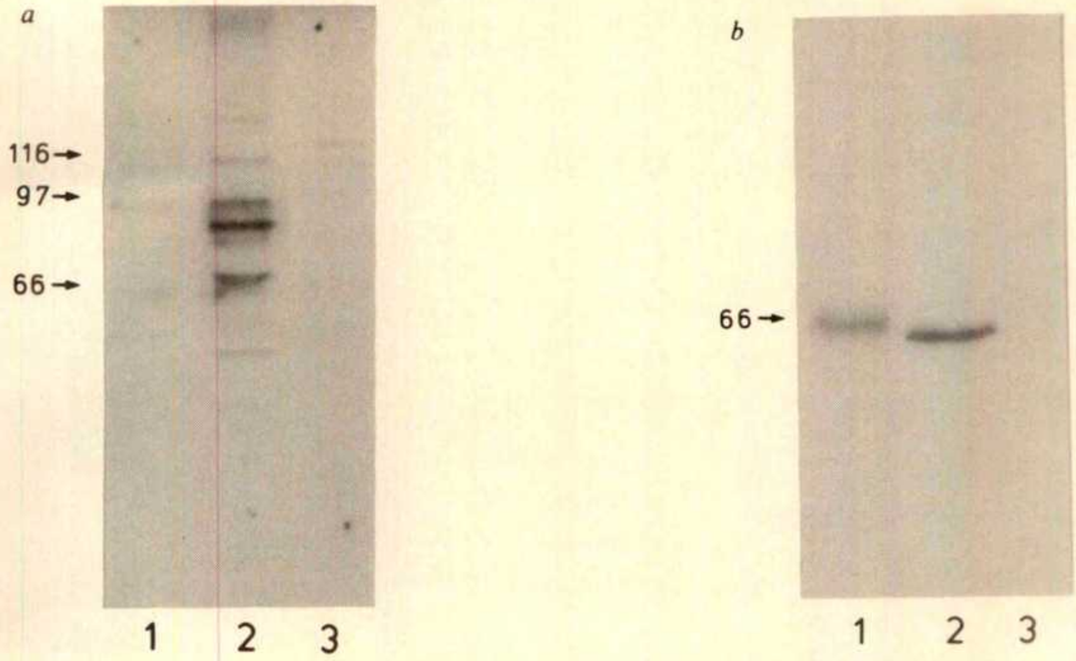

FIG. 1 *a*, RHEK-1 cells transfected with cloned EBNA-2, 100% positive by immunofluorescence staining, were incubated on immunoblots with the human polyvalent EBV-positive serum PG. A new band at 90K, corresponding to EBNA-2 in the B95-8 cells (lane 2), was detected (lane 1), but not in the untransfected RHEK-1 cells (lane 3). *b*, LMP-specific monoclonal antibody, S-12, detected a novel 63K band in the LMP-transfected RHEK-1 cells (lane 1) but not in RHEK-1 cells (lane 3). This band corresponded to the LMP of the EBV-transformed lymphoblastoid cell line, IB4, which was running slightly faster (lane 2).

METHODS. Cells were trypsinized and washed once in PBS before being sonicated for 3 × 10 s in a 50 mM sodium phosphate buffer pH 6.8 containing 2% SDS, 2 mM DTT, 5% glycerol and a pinch of bromophenol blue. The lysate was boiled for 5 min and applied to a 7.5% SDS-PAGE gel essentially as described by Laemmli[18]. After separation the proteins were routinely transferred to a nictrocellulose filter in a Bio-Rad mini-blot apparatus. The filters were stained with Ponceau S (Sigma) and the position of the molecular weight markers (Bio-Rad) was noted before they were destained and preabsorbed in PBS containing 5% non-fat dry milk. They were then incubated with serum or monoclonal antibodies overnight at 4 °C and exposed to antibodies conjugated to anti-IgG alkaline phosphatase antibodies conjugated to (Bio-Rad) for 2 h. After six washes in 0.5% Tween 20, the phosphatase substrate was added. IB4 and the EBV-negative B-cell lymphoma line BJAB were used as positive and negative controls respectively for the detection of EBNA-2 and LMP by immunoblotting (not shown). The S-12 monoclonal antibody directed against the C-terminal of LMP and a polyclonal EBV-positive human serum, PG were used for identifying LMP and EBNA-2.

weaker pattern of bands also appeared in the cells transfected with EBNA-2. The same antibody mixture reacted against only one band of about 42K in LMP-transfected cells (Fig. 2*c*). Anticomplementary immunofluorescence (ACIF) with EBNA-2-specific antibodies gave a brilliant, finely dispersed nuclear fluorescence with EBNA-2-transfected, but not LMP-transfected or control cells (Fig. 3).

The LMP-transfected cells changed to a more fibroblastic morphology with altered architecture and growth pattern. The flat and even cobblestone-like colonies that characterized keratinocytes cultured *in vitro* could not be recognized. Instead, the cells grew in tight, disorganized bundles that became partly multilayered and formed foci long before they reached confluence. The EBNA-2-transfected cells showed no detectable change compared to the untransfected cells. A similar change to fibroblastic morphology and piling-up in foci was previously observed in the same line after transfection with v-*ras* (ref. 6).

The EBV genome has been detected in the oropharynx and salivary glands[7] and virus-producing foci have been identified in the oral hairy leukoplakia of AIDS patients[8], but no epithelial tumors other than NPC are associated with EBV[9]. Six nuclear antigens (EBNA-1-6) and LMP are regularly expressed in virally transformed immortalized B-cell lines (LCL)[10]. Their function is not, or only incompletely, known. EBNA-1 is expressed in all EBV-infected cells. It binds to the origin of replication (*oriP*) and is required for maintenance of multiple viral genomes in an episomal form[11]. EBNA-2 is essential for B-cell activation, a prerequisite for B-cell immortalization[12]. The receptor-like structure of LMP has promoted speculation on its possible involvement in an autocrine circuit[13] and can transform established rodent fibroblast lines *in vitro*[14,15]. The transforming effect and the down-regulation of keratin expression in the LMP-transfected keratinocytes, together with the fact that LMP is only expressed in Burkitt's lymphoma after long periods of propagation *in vitro* and in parallel with a drift toward a more

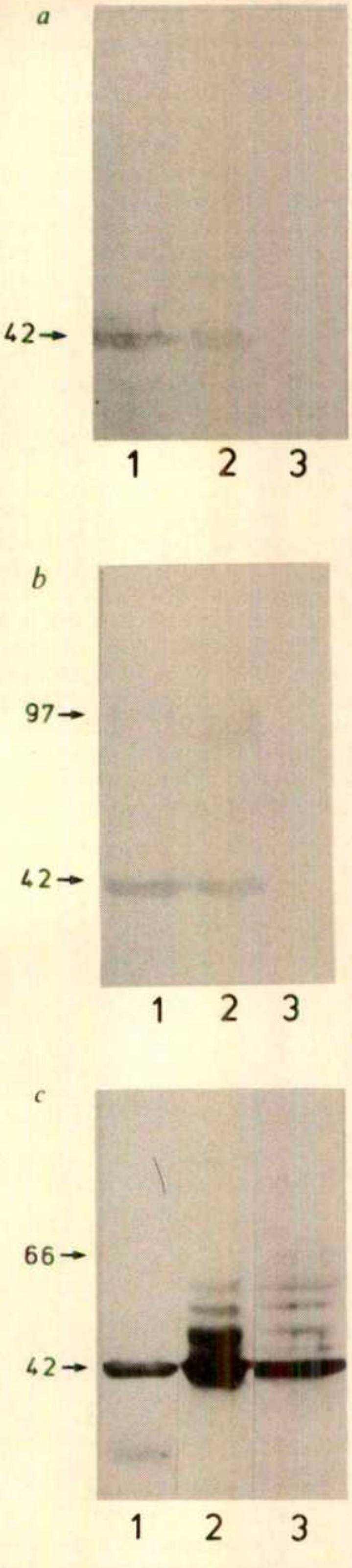

FIG. 2 Immunoblotting with the monoclonal antibodies PKK-1 (*a*) and PKK-2 (*b*) (Labsystem) against cytokeratins 19, 18 and 8 and against 19, 17, 16 and 7 respectively. Lane 3 shows the LMP-transfected RHEK-1 cells compared with RHEK-1 in lane 1 and the RHEK-1 cells tranfected with EBNA-2 in lane 2. *c*, Pattern of cytokeratins between 40 and 66K detected in the RHEK-1 (lane 2) and the EBNA-2-transfected RHEK-1 cells (lane 3) with the AE1/AE3 (Boehringer & Mann) pool of mixed monoclonal antibodies. The LMP-transfected RHEK-1 cells (lane 1) shows a down-regulation of cytokeratin expression.

METHODS. The immunoblotting is describing under Fig. 1. All antibodies were used in a dilution 1:500.

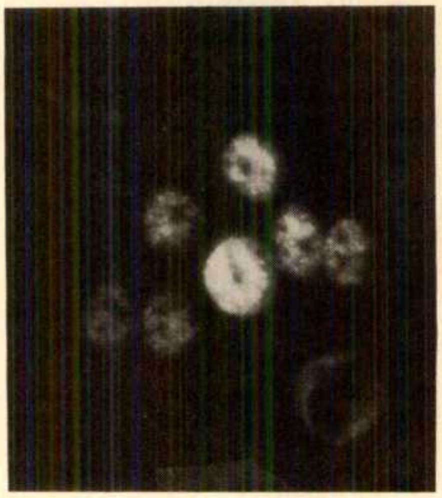

FIG. 3 Nuclear fluorescence of EBNA-2-transfected, uncloned RHEK-1 cells stained against an EBV-positive serum, pre-absorbed with EBNA-2-negative P3HR-1 cells.

METHODS. The RHEK-1/EBNA-2 cells were cultured in a tissue-chamber slide (LabTech) and fixed in −20 °C acetone/methanol (2/1) for 15 min. Three EBNA-2-antibody-negative and three EBNA-2-positive polyclonal sera were used. One of the latter (KF) was absorbed with P3HR-1 cells positive for EBNA-1, EBNA-3 and LMP and negative for EBNA-2. After incubation for 30 min at 37 °C in a wet chamber EBV-negative human complement was added and incubated at 37 °C for 20 min. Anti-B1C-FITC (DAKO) was added for 30 min at room temperature. The washed cells were counterstained for 5 min in Evans Blue.

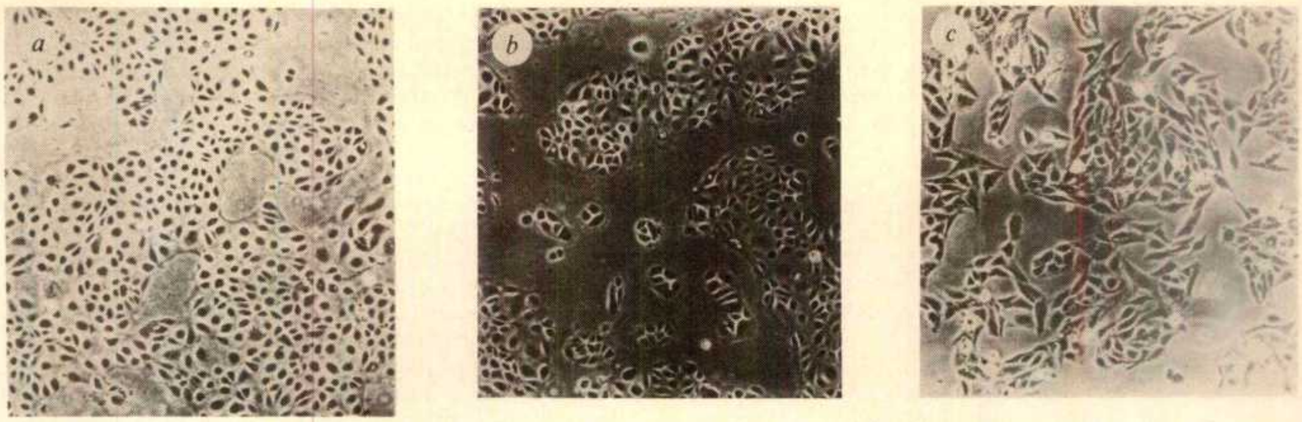

FIG. 4 *a*, RHEK-1 cells: primary foreskin keratinocytes immortalized with Ad12-SV40 hybrid virus. *b*, RHEK-1 cells transfected with the EBNA-2 gene, and showing no change in morphology. *c*, RHEK-1 cells transfected with the LMP gene. Note the change in morphology and growth in spindle-shaped bundles.

METHODS. Growth and maintenance medium consisted of RPMI with 10% fetal calf serum, hydrocortisone (2 μg ml^{-1}), penicillin and streptomycin. Cells transfected with EBNA-2 and LMP were kept on a selective medium containing mycophenolic acid (MPA) (10 μg ml^{-1}) and xanthine (100 μg ml^{-1}). Plasmids pJ132 and pJ144 carry the LMP and EBNA-2 genes, respectively, under the control of the SV40 early promoter. They were made by cloning a *Bgl*II-*Bam*HI fragment containing the SV40 enhancer and promoter sequence into the unique *Bam*HI site of plasmid pSV2gpt/*Bgl*II (ref. 19) resulting in plasmid pJ124 which allows transcription from both SV40 promoters in the same direction. pJ132 was made by cloning the LMP-encoding, 3,535-bp *Bam*HI-*Sac*II fragment of B95-8 EBV DNA[20] into the *Bam*HI site of pJ124 using *Bam*HI linkers. pJ144 was constructed by cloning the *Bgl*II fragment of pE-A6, encoding EBNA-2[21] into the *Bam*HI site of pJ124. The DNA was transfected by electroporation[22]. Cells were trypsinized and washed with PBS. DNA was added (10 μg per 5×10^6 cells) directly to the resuspended cell pellet together with 100 μl of HeBS buffer. The mixture was transferred into cuvettes, electroporated with 170 V at 960 μF and put on ice for 10 min. The cells were cultured in regular medium and selection started after 24 h by adding 5 μg ml^{-1} MPA and 50 μg ml^{-1} xanthine. The concentration of MPA and xanthine was doubled after a week and maintained subsequently at the same level. The cells were then cloned in a multichamber dish and tested for either LMP or EBNA-2 expressions.

LCL-like phenotype[16], raises the question of a role for LMP in the aetiology of NPC, a notoriously little differentiated or anaplastic tumour. This attractive hypothesis must be reconciled with the fact, however, that LMP is not detected in approximately 35% of NPC tumour biopsies[3]. The possibility that this could be due to technical artefacts involved in detecting LMP in tumour tissue must first be excluded. If the finding is real it may relate to the documented fact that LMP is highly immunogenic for T cells[17]. It is conceivable that LMP-negative tumours may represent a secondary immunoselected variant. Alternatively, LMP might not be essential for the genesis of the tumour but could provide an advantage for its progressions. □

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1. Klein, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **71,** 4737–4741 (1974).
2. Klein, G. in *The Epstein-Barr Virus* (eds Epstein, M. A. & Achong, B. G.) 339–350 (Springer, Berlin, 1979).
3. Fåhraeus, R. *et al. Int. J. Cancer* **42,** 329–338 (1988).
4. Moll, R. *et al. Cell* **31,** 11 (1982).
5. Sun, T.-T. *et al.* in *The Cancer Cells* vol 1 (eds Levin, A., Vaude Woude, G. F., Topp, W. & Watson, J.) 169–176 (Cold Spring Harbor, New York, 1984).
6. Rhim, J. S., Gilbert, J., Arnstein, P., Price, F., Sanford, K. & Aaronson, S. *Science* **227,** 1250–1252 (1985).
7. Sixbey, J. W., Nedrud, J. G., Raub-Traub, N., Hanes, R. A. & Pagano, J. S. *N. Engl. J. Med.* **310,** 1225–1230 (1984).
8. Greenspan, J. S. *et al. N. Engl. J. Med.* **313,** 1654–1571 (1985).
9. Andersson-Anvret, M., Forsby, N., Klein, G. & Henle, W. *Int. J. Cancer* **20,** 486–494 (1977).
10. Dillner, J. & Kallin, B. *Adv. Cancer Res.* **50,** 95–158 (1988).
11. Yates, J., Warren, N., Reisman, D. & Sugden, B. *Proc. natn. Acad. Sci. U.S.A.* **81,** 3806–3810 (1981).
12. Skare, J. *et al. J. Virol.* **55,** 268–297 (1985).
13. Mann, K. P. & Thorley-Lawson, D. *J. Virol.* **61,** 2100–2108 (1987).
14. Wang, D., Liebowitz, D. & Kieff, E. *J. Virol.* **62,** 2337–2346 (1988).
15. Baichwald, V. R. & Sugden, B. *Oncogene* **2,** 461–467 (1988).
16. Rowe, M. *et al. EMBO J.* **6,** 2743–2751 (1987).
17. Murray, R. *et al. J. Virol.* **62,** 3747–3755 (1988).
18. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
19. Rymo, L., Klein, G. & Ricksten, A. *Proc. natn. Acad. Sci. U.S.A.* **82,** 3435–3439 (1985).
20. Arrand, J. R. *et al. Nucleic Acids Res.* **9,** 2999–3014 (1981).
21. Ricksten, A., Svensson, C., Welinder, C. & Rymo, L. *J. gen. Virol.* **68,** 2407–2418 (1987).
22. Potter, H., Weir, L. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **81,** 7161–7165 (1984).

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Presentation of viral antigen controlled by a gene in the major histocompatibility complex

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WE describe a mutant human cell line (LBL 721.174)[1,2] that has lost a function required for presentation of intracellular viral antigens with class I molecules of the major histocompatibility complex (MHC), but retains the capacity to present defined epitopes as extracellular peptides. The cell also has a defect in the assembly and expression of class I MHC molecules[2,3,4], which we show can be restored by exposure of the cells to a peptide epitope. This phenotype suggests a defect in the association of intracellular antigen with class I molecules similar to that described for the murine mutant RMA-S (ref. 5), but in the present case the genetic defect can be mapped within the MHC locus on human chromosome 6.

The human mutant cell line LBL 721.174 (0.174) was selected from the B-cell line LBL 721 by exposure to gamma rays and sequential treatments with monoclonal antibodies against class I and class II molecules and complement[1]. It has a defect in assembly and transport of class I molecules that has resulted in complete loss of expression of B5, and 70–80% reduction in A2, at the cell surface[2,4]. These features resemble the phenotype of the murine mutant RMA-S[5], which has a selective defect in presentation of intracellular viral antigens to class I-restricted

T cells. We therefore looked for defects in antigen presentation in 0.174 cells.

We studied the ability of 0.174 cells to present an epitope (amino acids 58–68) of influenza matrix protein to a specific A2-restricted T-cell line (JM22) (ref. 6). The parental cells were lysed efficiently after infection with either a recombinant vaccinia virus that makes matrix protein (Fig. 1*b*), or influenza virus (data not shown). In contrast, the mutant 0.174 cells were not recognized in identical conditions (Fig. 1*a*). The lack of recognition of the infected 0.174 cells by JM22 T cells was not due to inefficient infection, as 0.174 and LBL 721 synthesized similar amounts of viral proteins after infection (data not shown).

Mutant cells, despite their reduced expression of A2 at the cell surface, required roughly 100-fold less peptide epitope than the wild-type cell to sensitize them for lysis by JM22 cytotoxic T lymphocytes (CTL) (Fig. 1*a–c*). These results showed that the defect in presentation in 0.174 was selective for intracellular antigen, and revealed an increased capacity of the mutant cell to present extracellular peptide.

To confirm that the A2 molecules of 0.174 were functional, and to establish that the mutation responsible for the loss of antigen presentation is on chromosome 6, we compared two derivatives of 0.174 obtained by fusion to the T lymphoma CEM[7]. The 0.174 × CEM hybrid (T1) expressed high levels of A2 and B5 derived from 0.174[7]. Its derivative (T2), selected for loss of both copies of human chromosome 6 provided by CEM, has the low class I expression phenotype of 0.174. This indicated that a *trans*-acting element encoded on CEM chromosome 6 enhanced class I expression in T1[7].

We found that T1 presented intracellular antigen, but T2 did not (Fig. 1*d*, *e*). These results placed the mutation responsible for the defect in antigen presentation in 0.174 cells on human chromosome 6. In addition they confirmed that this defect is not due to a mutation in A2 molecules. The mutant 0.174 bears a large homozygous deletion on the short arm of chromosome 6 between the DP alpha 2 locus and the complement gene cluster[8]. It is therefore likely that the gene(s) responsible for the defect in antigen presentation exist within this deleted region of the MHC.

To ensure that the defect in intracellular antigen presentation in the mutant was not limited to A2 and influenza matrix protein, T2 cells were transfected with several other class I genes and tested for presentation of epitopes of influenza virus that we have defined with synthetic peptides. In each case presentation of intracellular antigen was lost, whereas extracellular peptides were presented efficiently (table 1).

These findings show that the genetic defect in mutant 0.174 cells, like that in the murine mutant RMA-S[5], results in both loss of presentation of intracellular antigens and defective expression of class I molecules at the cell surface. These two traits could be consistent with a single gene defect by postulating that class I assembly and transport is facilitated by association with peptides derived from intracellular antigens[5].

The residual expression of A2 on 0.174, and the enhanced ability of the cells to present extracellular peptides, could be accounted for by assuming that a low level of assembly in the endoplasmic reticulum (ER), and expression of 'empty' A2 molecules at the cell surface, can occur in the absence of endogenous peptides. Murine class I glycoproteins encoded by transfected genes are similarly expressed at the cell surface in T2[9,10], but also do not present endogenous antigens to CTL (for example K^b in Table I).

By analogy with RMA-S[5], exposure of 0.174 or T2 cells to a sufficiently high concentration of extracellular peptides should enhance surface expression of class I molecules. Figure 2 shows that A2 expression at the 0.174 cell surface increased up to threefold after exposure to the peptides M58-68 or K62 (a more

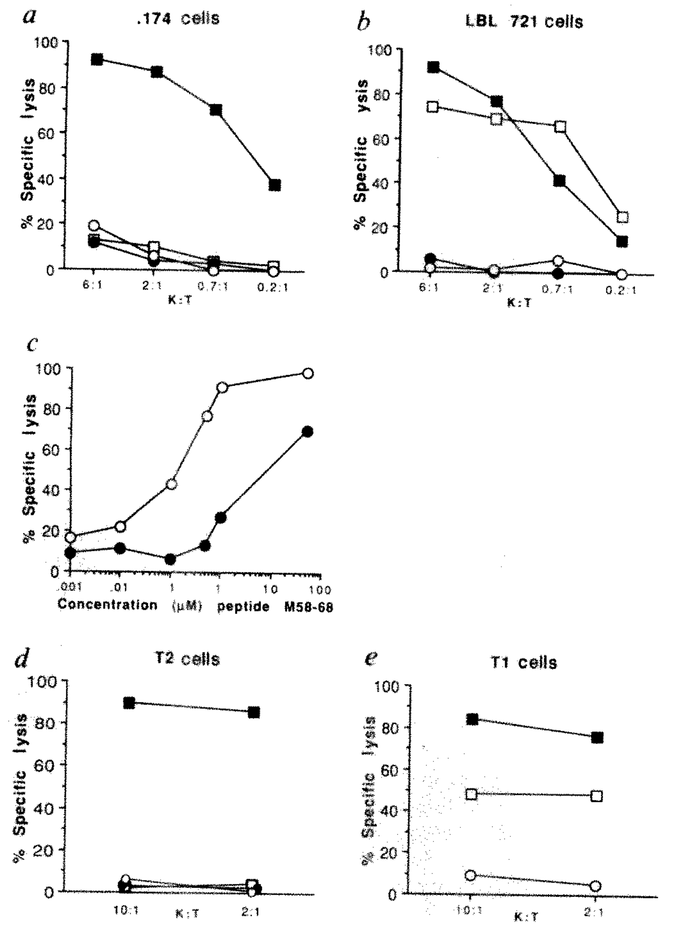

FIG. 1 Antigen presentation by 174 cells. *a*, *b*, JM22 T-cell line specific for the influenza matrix protein epitope 58–68 recognizes 0.174 cells treated with peptide M 58–68, but not infected with a recombinant vaccinia which makes matrix protein (M1-VAC). Target cells were: *a*, mutant 0.174, *b*, the wild-type LBL 721. Target cells were: □, infected with M1-vac; ■, treated with peptide M58–68; ●, infected with wild-type vaccinia virus; ○, untreated. *c*, The mutant 0.174 cells present peptide M58–68 more efficiently than wild-type LBL 721 cells. Target cells were: ○, 0.174 and ●, LBL 721 cells exposed to the peptide M58–68 at the concentrations shown. *d*, *e*, Fusion of 0.174 with CEM cells restores presentation of intracellular antigen. In *d*, target cells were T2 cells (0.174 × CEM selected for loss of CEM chromosomes 6); and in *e*, T1 (0.174 × CEM). Target cells were: □, T2 cells infected with M1-vac; ■, T2 cells treated with peptide M58–68; ●, T2 cells infected with wild-type vaccinia virus; ○, T2 cells untreated. In (*e*), target cells were: □, T1 cells infected with influenza virus X31; ■, T1 cells treated with peptide M58–68; ○, T1 cells untreated.

METHODS. JM22 is an influenza A virus-specific A2-restricted T-cell line which recognizes specifically the matrix 1 epitope 58–68 isolated as described[6]. Its activity was tested by Cr^{51} release assay on peptide treated (5×10^{-5} M for 1 h) or M1-vac infected (5 plaque-forming units per cell) or influenza X31 virus infected target cells[6]. In *c*, target cells were exposed to various concentrations of peptide M58–68 for 1 h, then washed twice and exposed to JM22 plated at the killer to target ratio (K:T) of 10:1. Cr^{51}-labelled cells were plated at 5×10^3 cells per well at the K:T ratios shown. Supernatants were collected after 4 h and specific lysis calculated as described[16].

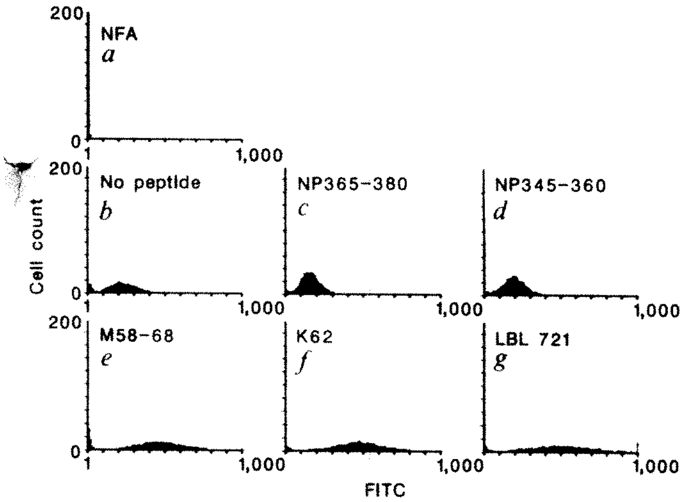

FIG. 2 Induction of A2 surface expression on 0.174 cells by peptide M58–68. *a*, Background staining 0.174 cells with no first antibody (NFA); *b*, basal level MA2.1-specific staining detected on untreated 0.174 cells; *c–f*, 0.174 cells stained with MA2.1 after treatment with the peptides shown; *g*, MA2-1-specific staining detected on wild type LBL 721 cells.

METHODS. Cells (5×10^5) were exposed to medium alone (RPMI/10% FCS) or medium containing peptides at the concentration of 10^{-4} M in a total volume of 1 ml for 12 h at 37 °C, then collected and stained by indirect immunofluorescence[5]. The first layer was MA2.1 (protein A-Sepharose purified and used at 5 μg ml^{-1}), and the second layer was FITC-labelled affinity-purified goat anti-mouse antibody (Sigma) at 1:60 dilution. Samples were analysed on an Ortho Cytofluorograf.

FIG. 3 Effect of peptide on conformation of A2 molecules detected by the MA2.1 antibody. *a*, Peptide induces a conformational change in the alpha-1 domain of the A2 heavy chain in 0.174 and T2 cells. Lane A, untreated 0.174 cells; B, 0.174 cells +NP345–360 (negative control); C, 0.174 cells +M58–68; D, 0.174 cells +K62; E, untreated LBL721 cells; F, untreated T2 cells; G, T2 cells +NP 345–360; H, T2 cells +M58–68; I, T2 cells +K62; J, untreated T1 cells. *b*, Peptide induces the conformational change in endo H-sensitive heavy chains. Samples were immunoprecipitated with MA2.1. Lanes A–D, untreated T2 cells; lanes E–H T2 cells treated with peptide NP 345–360; lanes I–L, T2 cells treated with peptide M58–68. Immunoprecipitated A2 molecules in lanes B, D, F, H, J, L were digested with endo H, and in lanes A, C, E, G, I, K, they were mock-digested. The position after digestion of endo H-resistant (R) and -sensitive (S) A2 molecules are marked on the right. Samples in lanes A, B, E, F, I, J were lysed after 15-min labelling, samples in lanes C, D, G, H, K, L were lysed after a 4-h chase in medium containing excess unlabelled methionine.

METHODS. *a*, 0.174, LBL 721, T2 and T1 cells were resuspended at 5×10^6 ml in medium (RPMI 1640/10% FCS) alone or containing peptides at 2×10^{-4} M for 5 h at 37 °C. They were then washed in 2 ml warm methionine-free medium and resuspended at 2×10^7 ml^{-1} in methionine-free medium containing peptides at the same concentration for a further hour. [^{35}S]methionine (120 μCi) was then added and the mixture incubated for 15 min at 37 °C. Labelled cells were washed in ice-cold PBS and resuspended in 0.5 ml lysis buffer as described[5]. Lysates were precleared with *Staphylococcus a* organisms overnight at 4 °C and immunoprecipitated[5] with purified MA2.1 antibody at a final concentration of 10 μg ml^{-1}. Reduced immunoprecipitates were examined by 12% SDS-PAGE which was fixed, stained, treated with Amplify (Amersham), dried and exposed to pre-flashed X-ray film. *b*, T2 cells were resuspended at 5×10^6 ml^{-1} in RPMI/10% FCS containing peptides at 2×10^{-4} M for 5 h. Aliquots of 2×10^7 T2 cells were resuspended in 1 ml methionine-free medium as above. After incubation for 1 h, 250 μCi [^{35}S]methionine was added for 15 min. The cells were then diluted to 5×10^6 ml^{-1} by addition of 3 ml chase medium (RPMI, 10% FCS, 4 mM unlabelled methionine, 10 mM HEPES) containing peptides at the same concentration. One half of the cells were collected immediately, the remainder incubated for 4 h at 37 °C. Samples were washed, lysed in 1 ml lysis buffer and precleared as before. Immunoprecipitation was with purified MA2.1 antibody at a final concentation of 10 μg ml^{-1} (ref. 5). The endo H treatment has been described[17].

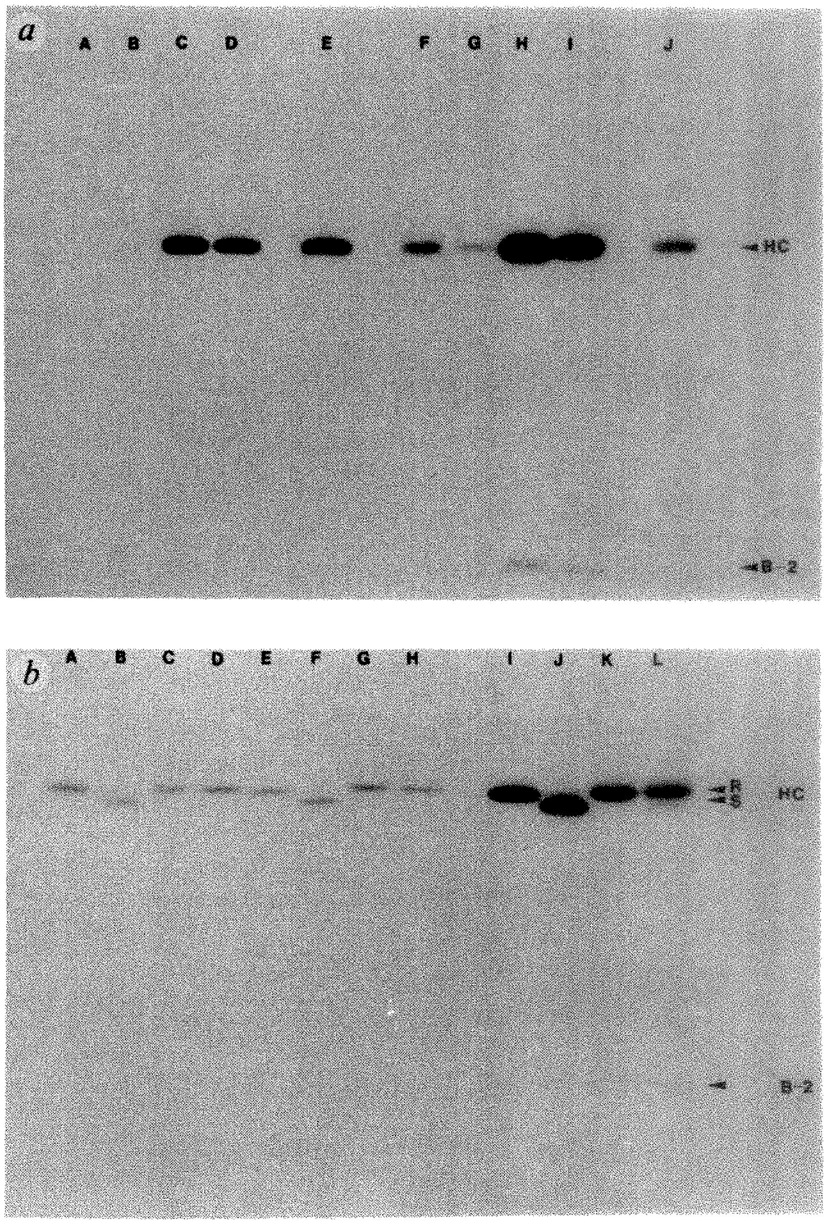

TABLE 1 Antigen presentation by T2 cells

Targets	Influenza-specific CTL restricted to Kb	 Aw68.1	 B27
	% Specific lysis		
X31 + T2-Kb	7	ND	ND
X31 + C1R-Kb	50	ND	ND
H17 + T2-Aw68.1	ND	6	ND
NP89-101 + T2-Aw68.1	ND	67	ND
H17 + C1R-Aw68.1	ND	49	ND
NP89-101 + C1R-Aw68.1	ND	66	ND
X31 + T2-B27	ND	ND	2
NP383-390 + T2-B27	ND	ND	40
X31 + C1R-B27	ND	ND	85
NP383-390 + C1R-B27	ND	ND	98

The defect in antigen presentation in T2 is not limited to presentation of antigens with A2. T2 cells, transfected with class I genes coding for H-2 Kb, Aw68.1 or B27 were tested for presentation of viral antigens to influenza-specific CTL after virus infection or exposure to peptides. T2 cells and the control cell line C1R (an A, B null mutant LBL cell line[9]) were transfected with Kb, Aw68.1 and B27 as described[9]. Kb-restricted cytotoxic T lymphocytes (CTL) were generated from C57BL mice primed by intranasal infection with X31 influenza virus as described[12], and restimulated *in vitro* with C1R transfected with Kb and infected with X31 virus. The epitope recognized by these CTL has not been defined. Aw68.1 restricted CTL (V.C. and A.T., manuscript in preparation) stimulated by E61-13-H17 influenza[13,14], and B27 restricted CTL stimulated by X31[15], recognize different epitopes mapped on the influenza nucleoprotein (NP) corresponding to the synthetic peptide indicated. The K to T ratio used was 10:1, and target cells were treated for 1 h with 5×10^{-5} M peptides. The per cent specific lysis was calculated as described[16]. ND, not determined.

soluble variant), and that this effect was peptide specific. The effect was blocked by treatment of the cells with brefeldin A (data not shown), implying that it depended on exit of class I molecules from the ER.

The effect of peptide on the conformation and transport of A2 molecules is shown in Fig. 3. Exposure to either peptide M58-68 or K62 (but not the control) increased the amount of A2 detected with MA2.1 (that binds folded A2 molecules associated with β-2microglobulin[11]) in both 0.174 and T2 cells (Fig. 3*a*). However, total A2 detected with a serum raised to the cytoplasmic domain, was not altered by treatment with peptides (data not shown). Figure 3*b* shows that the peptides induced this conformational change on A2 heavy chains that are endo H-sensitive (lanes A, B and E, F), but which become resistant over a 4 h chase period (lanes C, D and G, H). This implies that peptide acts on A2 heavy chains before their entry into the Golgi apparatus.

These findings show that the phenotype of 0.174 and T2 cells is closely related to that of the murine mutant RMA-S[5]. The phenotype of these cells may be explained by the lack of available peptides during assembly of class I molecules in the endoplasmic reticulum. This might be due to loss of transport of peptides from the cytosol[5], or possibly secondary to a defect in a peptide loading mechanism in the ER. Our results extend previous observations, and localize the mutation responsible for the loss of antigen presentation to the major histocompatibility complex on human chromosome 6. ☐

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1. DeMars, R., Chang, C. C., Shaw, S., Reitnauer, P. J. & Sondel, P. M. *Hum. Immun.* **11,** 77–97 (1984).
2. DeMars, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **82,** 8183–8183 (1985).
3. Shimizu, Y. *et al. Mol. cell. Biol.* **6,** 1074–1087 (1986).
4. Salter, R. D. & Cresswell, P. *EMBO J.* **5,** 943–949 (1986).
5. Townsend, A., Ohlen, C., Bastin, J., Foster, L. & Karre, K. *Nature* **340,** 443–448 (1989).
6. Gotch, F., Rothbard, J., Howland, K., Townsend, A. & McMichael, A. *Nature* **326,** 881–882 (1987).
7. Salter, R. D., Howell, D. N. & Cresswell, P. *Immunogenetics* **21,** 235–235 (1985.).
8. Whitehead, A. S., Colten, H. R., Chang, C. C. & DeMars, R. *J. Immun.* **134,** 641–641 (1985).
9. Alexander, J., Payne, J. A., Murray, R., Frelinger, J. & Cresswell, P. *Immunogenetics* **29,** 380–388 (1989).
10. Alexander, J., Payne, A. J., Shigekawa, B., Frelinger, J. & Cresswell, P. *Immunogenetics* **31,** 169–178 (1990).
11. Ways, J. P., Rothbard, J. B. & Parham, P. *J. Immun.* **137,** 217–222 (1986).
12. Townsend, A. R. M., Taylor, P. M., Mellor, A. L. & Askonas, B. A. *Immunogenetics* **17,** 283–294 (1983).
13. Baez, M., Palese, P. & Kilbourne, E. D. *J. infect. Dis.* **141,** 362–365 (1980).
14. Townsend, A. R. M. & Skehel, J. J. *J. exp. Med.* **160,** 552–563 (1984).
15. Huet, S. *et al. Int. Immun.* (in the press).
16. Townsend A. R. M. *et al. Cell* **44,** 959–968 (1986).
17. Owen, M. J., Kissonerghis, A. M. & Lodish, H. F. *J. biol. Chem.* **255,** 9678–9684 (1980).

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Circular DNA is a product of the immunoglobulin class switch rearrangement

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THE class of immunoglobulin is defined by the constant region of its heavy chain. When a B lymphocyte switches the class of heavy chain it produces, the constant region of μ-type heavy chain is replaced (reviewed in refs 1 and 2, and see ref. 3); this occurs through a DNA rearrangement that brings the gene segment encoding the new constant region close to the *VDJ* segment encoding the variable region. The pre-B-cell line 18–81, which switches from heavy chain μ to γ2b production in culture[4,5], occasionally abnormally rearranges the heavy chain locus so that DNA sequences between the switch regions of μ and γ2b are inverted[6]. Because looping-out is an intermediate step in generating an inversion, the switch rearrangement could occur by looping-out and deletion. Provided that recombination is reciprocal, this would produce a circle of DNA. Indeed, circular DNA molecules have been isolated as products of rearrangement among gene segments encoding the variable regions of the T-cell receptor[7–9] and of the immunoglobulin heavy chain[10,11] and light chain[11,12]. But whereas the breakpoints for the variable region rearrangement are precisely defined, the breakpoints for any given heavy chain class switch are scattered over a length of >6 kilobases, including both switch regions. We have now isolated circular DNA containing the sequences deleted by class-switching, thereby showing that the immunoglobulin heavy chain class switch occurs through looping-out and deletion.

A product of looping-out and deletion is a circle containing the constant region of μ-type heavy chain ($C\mu$) and the 3′ part of the μ switch region joined to the 5′ part of the switch (S) segment for the class to which the cell has switched (Fig. 1). These switch circles should be between 65 and 200 kilobases (kb) long, depending on the new class and on the exact location of the breakpoints.

As a likely source of switch circles, we used lipopolysaccharide-stimulated spleen cells, many of which had switched from μ to other heavy (H) chains[13–15]. After 3 days in culture, ~7% of the cells had switched to γ3, 8% to γ1, 3% to γ2b, and 7% to γ2a.

We purified circular DNA by the alkaline lysis method for mammalian cells[16,17]. Lysis of the cells under highly basic conditions results in denaturation of linear (chromosomal) DNA, whereas the strands of covalently closed circular DNA remain intertwined. Extensive shearing of the cell lysate promotes efficient strand separation of the chromosomal DNA. After neutralization and phenol extraction at high salt concentration, the single-stranded DNA that arose from denaturation of the chromosomal DNA separates at the interphase. Covalently closed circular DNA is recovered from the aqueous phase,

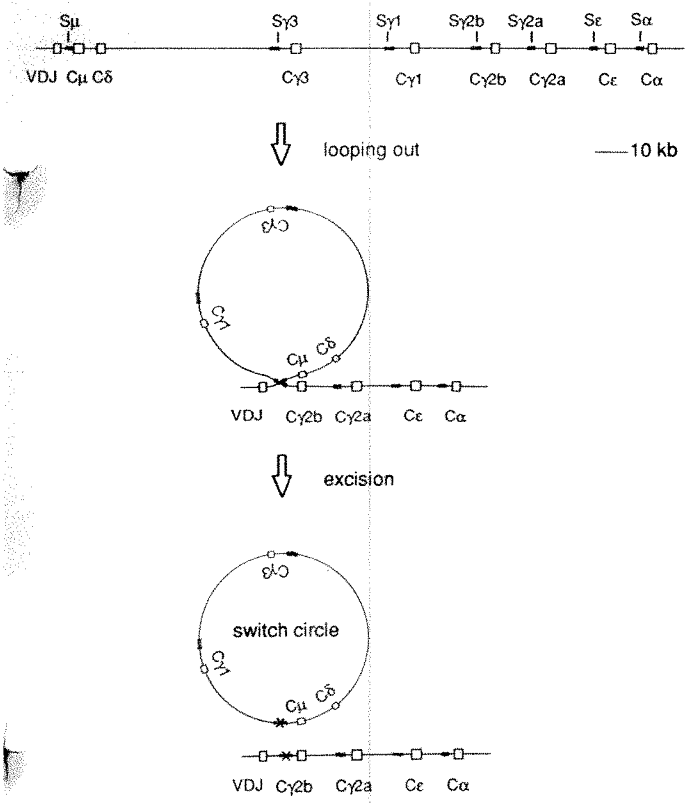

FIG. 1 Schematic representation of the H-chain locus showing organization of the constant gene segments, looping-out and deletion, and the resulting recombination products. In the mouse, the early immune response is dominated by IgM, which contains the H chain μ, and the later immune response is dominated by IgG3, IgG1, IgG2b, IgG2a, IgA and (rarely) IgE, which contain the H chains γ3, γ1, γ2b, γ2a, α and ε, respectively. Except for the constant region of δ-type *H* chain (*C*δ), each is preceded by a switch region of several kb within which the rearrangement breakpoint is usually found. X, switch recombination (break) point.

treated with RNase, and phenol-extracted again. Unlike caesium chloride density gradient centrifugation, this method allows isolation of large circles, including non-supercoiled ones, and takes only a few hours.

On Southern blots of circular DNA that had been digested with various restriction enzymes, we identified fragments that must be present in the switch circles. These fragments could not have been isolated from chromosomal DNA. Figure 2*a* shows a restriction map of the joining segments of the *H* locus (*J_H*) and *C*μ locus, which is needed to interpret the Southern blots (Fig. 2*b* and *c*). Figure 2*b* demonstrates the presence of *C*μ in the switch circle preparation. When hybridized with a *C*μ probe, the *Hin*dIII digest gave a 2.2-kb band and a 1.2-kb band in the circular DNA (lane 4), just as in the germ-line configuration (kidney) DNA (lane 3). Although at least 10 times more kidney DNA was applied to the gel, the bands of the circle preparation

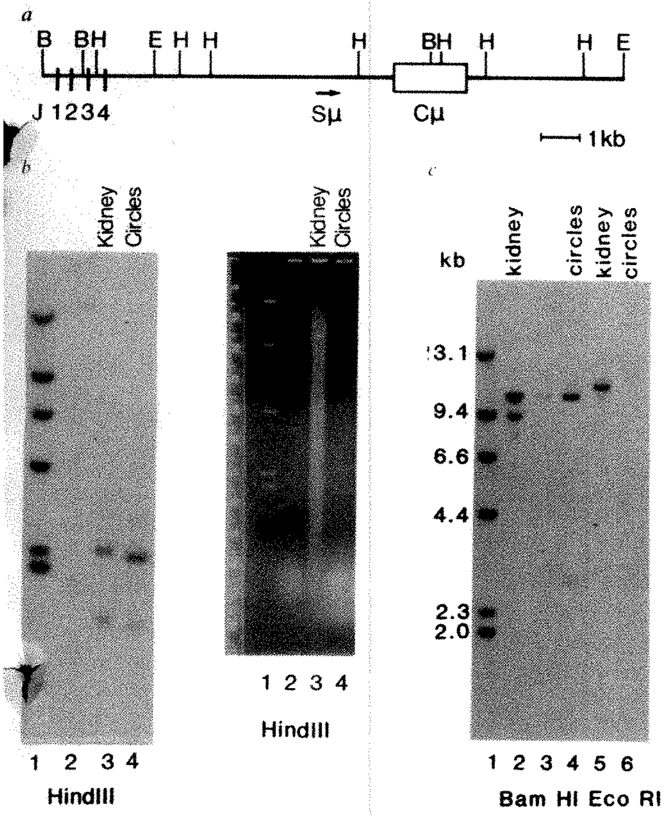

FIG. 2 Identification of the switch circles. *a*, Restriction enzyme map of embryonic BALB/c mouse DNA that includes the *J_H* and *C*μ gene segments. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII. *b*, Left: Southern blot analysis of kidney and circular DNA, digested with *Hin*dIII. The hybridization probe was *C*μ, a μ-gene complementary DNA fragment covering the first three exons. (The kidney DNA moves a little slower in the gel, a common occurrence in horizontal gels when excess DNA is loaded.) Lane 1, size marker. Right: ethidium bromide staining of the gel shown on the left. *c*, Southern blot analysis of kidney and circular DNA digested with *Bam*HI lanes 2, 4 and *Eco*RI lanes 5, 6. The hybridization probe was *C*μ. Lane 1, size marker. Spleen cells of C57BL/6 mice were grown in RPMI medium containing 50 μg ml^{-1} lipopolysaccharide at a density of 2.5×10^5 cells ml^{-1}. On day 3, production of immunoglobulin isotypes was checked by membrane immunofluorescence. Circular DNA from 1.6×10^9 cells was isolated and dissolved in buffer (800 μl). This solution (50 μl, or the equivalent of 1×10^8 cells) was digested with restriction enzyme and loaded onto an agarose gel for Southern blot analysis. Chromosomal DNA (10 μg, or the equivalent of 1×10^6 kidney cells) was loaded. Because there are two *C*μ copies per genome, this amount of DNA represents 2×10^6 copies of *C*μ. Circular and chromosomal DNA gave *C*μ bands of equal intensity. Thus, the circular DNA isolated from 1×10^8 spleen cells contains about 2×10^6 copies of *C*μ, or one switch circle per 50 cells. As only 30% of the spleen cells in culture had switched in isotype, this means that we could isolate a switch circle from 1 of 15 switched cells.

were as strong as those in the kidney DNA. Therefore, the $C\mu$ sequences in the circles could not all be due to chromosomal contamination unless preparation enriched for $C\mu$ sequences, an unlikely possibility. On the Southern blot shown in Fig. 2*c*, the *Bam*HI digest of kidney DNA (lane 2) gave a 9.4-kb band and an 11.2-kb band. The 9.4-kb band includes the sequences of *JH3* and *JH4*, as well as part of $C\mu$, whereas the 11.2-kb band covers the part of $C\mu$ 3′ to the *Bam*HI site in $C\mu$. The circular DNA prepared from lipopolysaccharide-stimulated spleen cells (lane 4) shows the 11.2-kb band, which again confirms the presence of $C\mu$ in the switch circles. The lack of the 9.4-kb fragment in the *Bam*HI-digested circular DNA is also predicted for switch circles because the germ-line sequences 5′ to $S\mu$ are replaced by the sequences 5′ to the switch region of the other classes (Fig. 1). Because there are six other classes to which the μ-gene can switch, and because the breakpoints will be scattered over the switch regions and sometimes beyond, we expect the 9.4-kb band to be replaced with a smear. Indeed, a smear is present in lane 4, above the 11.2-kb band. If the 11.2-kb band were of chromosomal origin, the 9.4-kb band should also be present, albeit with half the intensity of the germ-line band in lane 2. (We assume that half of the spleen cells have rearranged their *VD* segment to *JH1* or *JH2*, and half to *JH3* or *JH4*. Rearrangements to *JH1* or *JH2* do not alter the *Bam*HI site 3′ to *JH2*, thus preserving the 9.4-kb fragment; rearrangements to *JH3* or *JH4* delete the *Bam*HI site 3′ to *JH2*.) Further evidence that the circular DNA is not contaminated with chromosomal DNA is given by the *Eco*RI digest in Fig. 2*c*. The 13.5-kb germ-line fragment (lane 5) is absent in the circular DNA (lane 6). The chromosomal *Eco*RI fragment containing $C\mu$ should be the same, whether derived from germ-line or rearranged DNA. Instead of the 13.5-kb band, a smear of bands larger than 13.5 kb is present in the circular DNA (lane 6). This is expected for the same reasons as those given for the *Bam*HI digest. Southern blot analyses of DNA digested with *Bgl*II and *Xba*I led to the same conclusions.

From the Southern blot analyses we estimate that we isolated switch circles from 1 of 15 switched cells (for calculation see Fig. 2). If the average clone size of switched cells is four to eight (two to three generations), then we recovered 0.25–0.5 circles per class switch event. In view of the losses to be expected from degradation *in vivo* and during isolation, this is a high yield. If the circles do not replicate, the yield is consistent with a circle arising from every H class-switch rearrangement.

We think that the circles generated by a switch to γ2b do not replicate as episomes. In clones of a pre-B-cell line in which both alleles have switched to γ2b (ref. 6), no $C\mu$ sequences were detected by the polymerase chain reaction. If the circles replicated at the rate of the chromosomes, most of the γ2b-producing cells should contain at least one $C\mu$ segment.

We constructed a partial library of the *Eco*RI-digested switch circles in λ EMBL4 phage. On screening about 10^6 phages with a $C\mu$ probe we obtained 18 clones. We are aware that this procedure selects for a certain size-range of insert and probably excludes many fragments that contain breakpoints. As reasoned above, the breakpoints must be present on *Eco*RI fragments containing $C\mu$. The restriction maps of the DNA of the six different phage inserts analysed indicate that none was derived from chromosomal DNA (Fig. 3). Of five λ-inserts, we subcloned those fragments where we suspected the breakpoints to be, and sequenced parts of them. Four fragments had $S\gamma 2b$ joined to $S\mu$ sequences in a 5′-3′ orientation (reciprocal to the 5′$S\mu$-3′$S\gamma 2b$ configuration of the γ*2b* gene on the chromosome), and one fragment had $S\gamma 3$ joined to $S\mu$ (Fig. 4). Therefore, as predicted for the looping-out and deletion mechanism, the deleted DNA must have joined its ends to form a circle. The 5′ sequence of the $S\gamma 2b$ clones is 94% concordant with a 210-base pair (bp) segment of a published part of the $S\gamma 2b$ sequence MUSIGCD23 (GenBank accession number J00456; ref. 18). The 3′ sequence is 97% concordant with a 270-bp segment of the published $S\mu$ sequence MUSIGCD09 (GenBank accession no. J00442; ref. 19). Also, 144 bp of the 5′ part of the fifth fragment are 98% concordant with the $S\gamma 3$ sequences MUSIGHAL (GenBank accession no. J00483; ref. 18) and MUSIGHANA (GenBank accession no. M12182; W. Dunnick, unpublished observations), whereas its 3′ sequence is part of $S\mu$. We do not know whether the discrepancies reflect polymorphism (the GenBank sequences are derived from the BALB/c mouse strain, our circle sequences from the C57BL/6 strain) and/or sequencing errors. Although more spleen cells had switched to other γ-subclasses than to γ*2b*, four out of five switch-recombination sequences joined to $S\mu$ were from $S\gamma 2b$. This is probably due to our selection of *Eco*RI fragments for the library.

In principle, chimaeric switch regions, that is, 5′$S\gamma$-3′$S\mu$, could also be generated by recombination between sister chromatids or homologues. In these cases, however, the chimaeric switch regions would be located on chromosomal DNA, not on circles. Nevertheless, as the following calculation demonstrates, our results do not depend on any *a priori* estimate of the purity of our preparation of circular DNA. Immediately after the putative unequal crossing-over event, half, at most, of the $S\mu$ regions would be chimaeric. This fact is not altered by growth of the cells after the switch. Because no more than 30% of our cells switched, no more than 15% of the switch regions

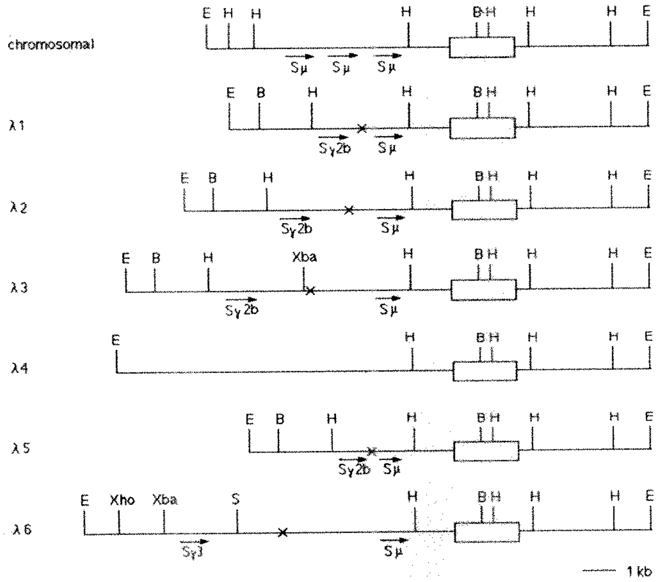

FIG. 3 Restriction maps of the chromosomal μ-locus and of 6 of the 18λ-clones containing inserts with the breakpoints from switch circles. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sph*I; Xho, *Xho*I; Xba, *Xba*I. X, putative breakpoints. The arrows at the $S\gamma 2b$, $S\gamma 3$ and $S\mu$ sequences show their original orientation at the *H* locus.

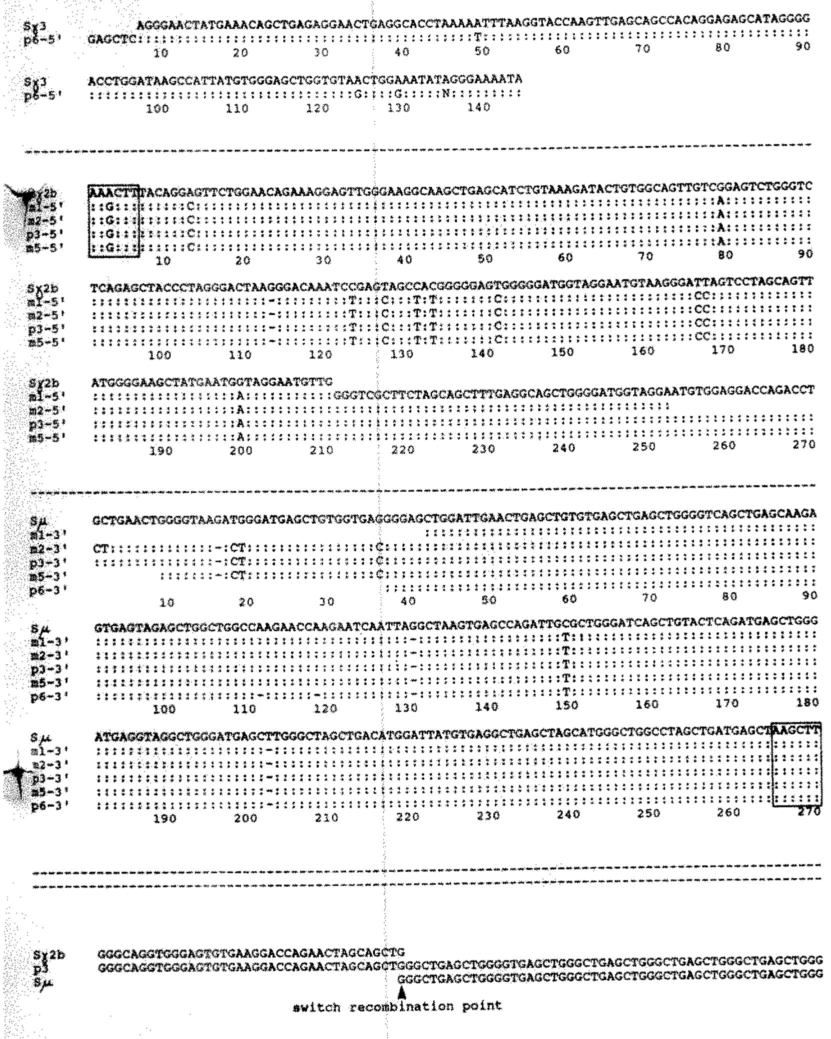

FIG. 4 *a*, Sequences of the 5′ and 3′ ends of five switch-circle fragments that contain the switch recombination points. Clones m1, m2 and m5 contain *Hin*dIII fragments of the λ-phages λ1, λ2 and λ5, respectively, subcloned into M13mp19; clone p3 contains a *Hin*dIII fragment of λ3 in pGem3, and pGem clone p6 contains the *Eco*RI–*Hin*dIII fragment of λ6. The sequences were aligned with a *S*γ*3* switch-region sequence (MUSIGHAL) or with a *S*γ*2b* sequence (MUSIGCD23) and with part of the *S*μ sequence (MUSIGCD09). :, base identity; —, a gap. Nucleotides 211–270 of the *S*γ*2b* sequence were not present in the GenBank. The *Hin*dIII restriction sites AAGCTT, that were used for subcloning, are boxed. *b*, Sequence of the switch recombination (break) point of clone p3. The 5′ part is identical to the *S*γ*2b* sequence MUSIGCD24 (GenBank accession no. J00457; ref. 18), the 3′ part represents the *S*μ consensus sequence, GAGCTGGGG/CT.

would be chimaeric. Even if the putative circular DNA were entirely linear the probability of our isolating six chimaeric out of a total of six switch regions would be 0.15^6, or ~0.00001. So it is reasonable to conclude that the chimaeric switch regions that we isolated are derived from circular DNA.

The existence of switch circles is evidence that looping-out and deletion occur during the heavy chain class-switch in mitogen-stimulated B lymphocytes. By itself, this does not exclude the possibility that two other proposed mechanisms might also achieve the same result: unequal exchange between sister chromatids[20,21] and between homologues. Illegitimate recombination between the switch regions and regions on other chromosomes must occur, albeit at a low frequency; for example, oncogenes are translocated to the immunoglobulin locus[22]. Class switching of a randomly integrated μ-transgene has also been reported[23], implying interchromosomal recombination, or *trans*-splicing of RNA. But we have excluded the possibility that either the exchange between sister chromatids[24] or the exchange between homologues[6,24] makes any substantial contribution to the heavy chain class-switch in a pre-B-cell line. Also unequal exchange between homologues has been excluded by the fact that the V and C region allotypes of a given immunoglobulin are both encoded by one homologue in the rabbit[25–28] and in the mouse[29]. We also note that, because in the mouse the order on the chromosome is centromere, *C*, *V* (ref. 30), recombination between homologues would create switched cells, half of which would contain two *C*μ segments on the silent homologue; the other half of switched cells would be μ-γ double producers with identical V regions on each isotype. These types of cells have not been demonstrated, which, in view of the many hybridomas analysed, is another strong argument against this mechanism making a significant contribution to the switch process.

As in the deletional joining of the *V*, *D*, and *J* segments of the T-cell receptor and immunoglobulin genes, the switch recombination includes a reciprocal ligation resulting in circular DNA. It has been proposed that circles are less recombinogenic with respect to chromosomal integration than linear molecules and thus minimize potential mutagenic damage[31]. ☐

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1. Shimizu, A. & Honjo, T. *Cell* **36,** 801–803 (1984).
2. Marcu, K. B. *Cell* **29,** 719–721 (1982).
3. Shimizu, A., Takahashi, N., Yaoita, Y. & Honjo, T. *Cell* **28,** 499–506 (1982).
4. Burrows, P. D., Beck, G. B. & Wabl, M. R. *Proc. natn. Acad. Sci. U.S.A.* **78,** 564–568 (1981).
5. Burrows, P. D., Beck-Engeser, G. B. & Wabl, M. R. *Nature* **306,** 243–246 (1983).
6. Jäck, H.-M., McDowell, M., Steinberg, C. M. & Wabl, M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1581–1585 (1988).
7. Fujimoto, S. & Yamagishi, H. *Nature* **327,** 242–243 (1987).
8. Okazaki, K., Davis, D. D. & Sakano, H. *Cell* **49,** 477–485 (1987).
9. Okazaki, K. & Sakano, H. *EMBO J.* **7,** 1669–1674 (1988).
10. Abe, M. & Shiku, H. *Nucleic Acids Res.* **17,** 163–170 (1989).
11. Toda, M., Hirama, T., Takeshita, S. & Yamagishi, H. *Immun. Lett.* **21,** 311–316 (1989).
12. McCormack, W. T. *et al. Cell* **56,** 785–791 (1989).
13. Kearney, J. F. & Lawton, A. R. *J. Immun.* **115,** 671–681 (1975).
14. Pernis, B., Forni, L. & Luzzati, A. L. *Cold Spring Harb. Symp. quant. Biol.* **41,** 175–183 (1976).
15. Wabl, M. R., Forni, L. & Loor, F. *Science* **199,** 1078–1080 (1978).
16. Griffin, B. E., Björck, E., Bjursell, G. & Lindahl, T. *J. Virol.* **40,** 11–19 (1981).
17. Carroll, S. M. *et al. Molec. cell. Biol.* **7,** 1740–1750 (1987).
18. Kataoka, T., Miyata, T. & Honjo, T. *Cell* **23,** 357–368 (1981).
19. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286,** 676–683 (1980).
20. Obata, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 2437–2441 (1981).
21. Tilley, S. A., & Birshtein, B. K. *J. exp. Med.* **162,** 675–694 (1985).
22. Altiok, E. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 6333–6337 (1989).
23. Durdik, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 2346–2350 (1989).
24. Wabl, M., Meyer, J., Beck-Engeser, G., Tenkhoff, M. & Burrows, P. D. *Nature* **313,** 687–689 (1985).
25. Kindt, T. J., Mandy, W. J. & Todd, C. W. *Biochemistry* **9,** 2028–2032 (1970).
26. Landucci Tosi, S., Mage, R. G. & Dubiski, S. *J. Immun.* **104,** 641–647 (1970).
27. Knight, K. L. & Hanly, W. C. *Contemp. Top. molec. Immun.* **4,** 55–58 (1975).

28. Pernis, B. *et al. Immunochemistry* **10,** 281–285 (1973).
29. Gearhart, P. J., Hurwitz, J. L. & Cebra, J. J. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5424–5428 (1980).
30. Seldin, M. F., Howard, T. A. & D'Eustachio, P. *Genomics* **5,** 24–28 (1989).
31. Alt, F. W. & Yancopoulos, G. D. *Nature* **327,** 189–190 (1987).

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Telomere–telomere recombination provides an express pathway for telomere acquisition

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DNA termini from *Tetrahymena* and *Oxytricha*, which bear C_4A_2 and C_4A_4 repeats respectively, can support telomere formation in *Saccharomyces cerevisiae* by serving as substrates for the addition of yeast telomeric $C_{1-3}A$ repeats. Previously, we showed that linear plasmids with 108 base pairs of C_4A_4 DNA (YLp108CA) efficiently acquired telomeres, whereas plasmids containing 28–64 base pairs of C_4A_4 DNA also promoted telomere formation, but with reduced efficiency[1]. Although many of the C_4A_4 termini on these plasmids underwent recombination with a C_4A_2 terminus, the mechanism of telomere–telomere recombination was not established[1–3]. We now report the sequence of the C_4A_4 ends from the linear plasmids. The results provide strong evidence for a novel recombination process involving a gene conversion event that requires little homology, occurs at or near the boundary of telomeric and non-telomeric DNA, and resembles the recombination process involved in bacteriophage T4 DNA replication.

Figure 1*a* shows the structure of linear plasmids before and after introduction into yeast (before transformation, there are no detectable C_4A_2 or C_4A_4 sequences in yeast[1]). We were able to distinguish yeast transformants where recombination at the C_4A_4 end had occurred, and those that did not undergo recombination, by Southern hybridization (Table 1). C_4A_4 termini were cloned and the junction between telomeric and non-telomeric DNA was determined. In each recombinant, more than 180 base pairs (bp) of C_4A_2 sequences were found at the C_4A_4 end (Fig. 2*c*); the polarity of the transferred C_4A_2 sequences was the same as that of the C_4A_4 repeats (Fig. 2*b*); and there was no detectable decrease in the amount of C_4A_2 DNA at the C_4A_2 end (data not shown).

These results have important implications for the mechanism of telomere–telomere recombination. First, the fact that a long stretch of C_4A_2 DNA is transferred, an amount indistinguishable from the amount of uninterrupted C_4A_2 DNA at the C_4A_2 end (Fig. 1*b*), argues that initiation (or resolution) of recombination at the C_4A_2 end must have occurred near or at the junction between the C_4A_2 repeats and non-telomeric DNA (Fig. 3). These data rule out models[1,2] in which recombination results from the transfer of repeats between nicks in the C_4A_2 strand (Fig. 1*b*) which predict that only short tracts of C_4A_2 should be transferred.

Second, these results rule out the possibility that transfer occurred by the resolution reaction. In this model[3,4], a C_4A_4 and a C_4A_2 end ligate together followed by breakage within the C_4A_2 sequences. Contrary to the observed results (Fig. 2*b*), the resolution reaction predicts that the orientation of the transferred C_4A_2 repeats would be inverted relative to that of the C_4A_4 end repeats[1,3].

Third, the fact that there is no detectable decrease in the number of C_4A_2 repeats at the C_4A_2 end indicates that recombination usually occurred by non-reciprocal recombination.

Intramolecular reciprocal recombination would result in loss of >180 bp of C_4A_2 DNA from the C_4A_2 end, a result that is

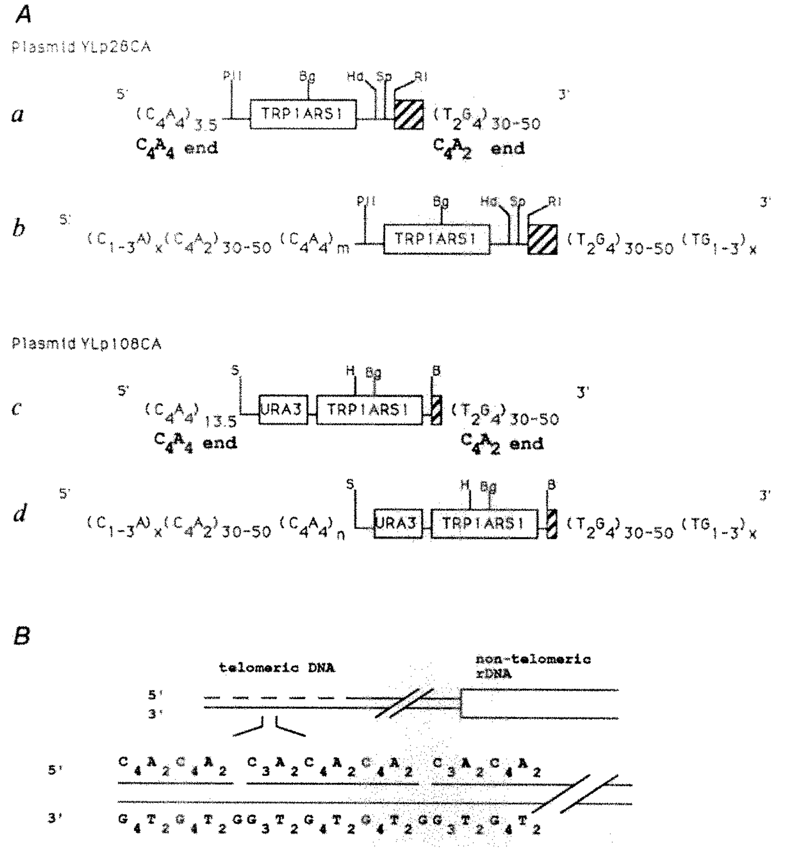

FIG. 1 *A*, Structures of plasmids YLp28CA and YLp108CA before (*a*, *c*) and after (*b*, *d*) introduction into yeast. One strand is represented in the 5′ to 3′ orientation (left to right). Construction of the linear plasmids has been described[1]. Plasmids YLp28CA and YLp108CA bearing 28 and 108 bp of C_4A_4 repeats, respectively, at the 'C_4A_4 end' and ~300 bp of C_4A_2 repeats at the 'C_4A_2 end' were introduced by transformation[21] into yeast. The structure of plasmid DNA in individual transformants carrying linear plasmids was determined by restriction enzyme mapping and Maxam and Gilbert DNA sequencing[22]. As expected, all linear plasmids acquired yeast $C_{1-3}A$ repeats at each end. Many (90%) plasmids had also undergone recombination with other termini as demonstrated by the transfer of tracts of C_4A_2 repeats to the C_4A_4 end. Sometimes (~50% of sequenced plasmids), the remaining C_4A_4 end length $(C_4A_4)_m$ [or $(C_4A_4)_n$] was less than 28 bp (or 108 bp) (see Table 1). The length of $(C_{1-3}A)_x$ or $(TG_{1-3})_x$ is ~300 bp. The hatched box indicates the C_4A_2-adjacent unique sequence from *Tetrahymena* rDNA. Abbreviations: Bg, *Bgl*II; B, *Bam*HI; H, *Hin*dIII; PII, *Pvu*II; RI, *Eco*RI; S, *Sal*I; Sp, *Sph*I. *B*, Structure of the terminus of *Tetrahymena* extrachromosomal rDNA molecules. These termini bear an average of 300 bp of C_4A_2 sequence. Specific single-base gaps occur every 12–18 nucleotides in the most terminal 100 bp of the molecule[23]. The G-rich strand is extended as a 12 to 16 nucleotide single-strand tail on at least some termini[24].

TABLE 1 Sequences at C_4A_4-end junctions

Yeast transformant	No. bp C_4A_4 retained	No. bp C_4A_2 transferred	Approximate bp $C_{1-3}A$	Sequence at the junction
YLp108CA (without transfer)				
1	108	0	300	ACACAT$_2$C$_3$ C$_4$A$_4$
2	84	0	300	CACACA C$_4$A$_4$
3	76	0	400	CACACA C$_4$A$_4$
4	36	0	300	CCCACA C$_4$A$_4$
YLp40CA				
1	36	>180	300	C$_4$A$_2$ C$_4$A$_4$
2	36	>180	300	C$_4$A$_2$ C$_4$A$_4$
3	12	>180	300	C$_4$A$_2$ C$_4$A$_4$
YLp28CA				
1	20	>240	380	C$_4$A$_2$ C$_4$A$_4$
2	12	>240	270	C$_4$A$_2$ C$_4$A$_4$
3	28	>240	270	C$_4$A$_2$ C$_4$A$_4$
4	28	>240	270	C$_4$A$_2$ C$_4$A$_4$

Linear plasmids with 108, 40, or 28 bp of cloned C_4A_4 DNA at the C_4A_4 end were introduced and propagated in yeast (see Fig. 1). The C_4A_4-end telomeres of plasmids YLp108CA and YLp28CA were cloned from various yeast transformants and sequenced as described (Fig. 2). The C_4A_4-end telomeres of plasmid YLp40CA were cloned in the same way as for YLp28CA. For each yeast transformant, two independent *Escherichia coli* telomere clones were sequenced. In each case, the amount of C_4A_4 DNA retained and the junction sequence between C_4A_4 and $C_{1-3}A$ (or C_4A_2) were identical between the two clones. Plasmids that underwent recombination obtained over 180 bp of C_4A_2 DNA at the C_4A_4 end before the addition of $C_{1-3}A$. The approximate length of added $C_{1-3}A$ sequence was estimated by agarose gel electrophoresis or in some cases determined by DNA sequencing. Sequences at the junction between the C_4A_4 DNA and the added $C_{1-3}A$ or C_4A_2 DNA are shown in the 5′ to 3′ orientation (left to right) running from the end towards the central portion of the linear plasmids.

never observed. Although 20% of the recombinants had C_4A_2 and C_4A_4 DNA on both ends, which is the product expected for reciprocal intramolecular recombination, even these molecules have no detectable loss of C_4A_2 DNA at the C_4A_2 end[1]. We cannot rule out the possibility that some recombinants are the result of reciprocal recombination between two different plasmid molecules. But we never recovered the reciprocal product of inter-molecular recombination in the same cell despite the fact that as long as this product has ~28 bp of telomeric DNA remaining at the C_4A_2 end, it should be replication-competent. Moreover, since reciprocal recombination will result in a reduction in the amount of C_4A_4 DNA at the C_4A_4 end, inter-molecular reciprocal recombination cannot account for the two YLp28CA transformants that retained the full 28 bp of C_4A_4 DNA after recombination. Recombination on these plasmids must have occurred by a non-reciprocal event.

When linear plasmid DNA is introduced into yeast, its ends are believed to be subject to exonucleolytic degradation. DNA sequencing demonstrates that even termini bearing telomeric repeats are subject to degradation: 5 of 7 of the sequenced C_4A_4 ends that had acquired C_4A_2 repeats (and 3 of 4 termini that had not acquired C_4A_2 repeats; see below) had fewer C_4A_4 repeats than the input plasmid (Table 1). Two termini that had undergone recombination retained only 12 bp of C_4A_4 DNA. Therefore, telomere–telomere recombination must require very little homology (no more than 28 bp and possibly as few as 12 bp). In contrast, for more conventional yeast sequences, 26 bp of perfect homology supports very little gene conversion (10^{-10} events per generation)[5].

We also examined the termini of plasmids that did not recombine. YLp108CA did not undergo recombination in 4 out of 19 transformants[1]. DNA sequencing of cloned C_4A_4 termini from these transformants revealed that no C_4A_2 repeats were transferred to the C_4A_4 end. These results confirm our earlier results[6] that C_4A_4 repeats are themselves suitable substrates for $C_{1-3}A$ addition in yeast. The junction between C_4A_4 DNA and $C_{1-3}A$ DNA was determined for all four termini (Table 1). In one case, all of the C_4A_4 repeats on the input plasmid were retained and the $C_{1-3}A$ repeats were added directly to the five bases of non-telomeric DNA that are the remnant of the restriction enzyme recognition site used to generate the C_4A_4 end (Table 1; Fig. 2*a*). This result demonstrates that telomeric DNA need not be at the end of a DNA molecule to be utilized for telomere formation.

The same conclusion was reached by others from restriction enzyme digestion of yeast linear plasmid DNA[4]. In this earlier study, the stretch of telomeric DNA (75 repeats of alternating CA sequence) was 66 bp from the end of the input linearized plasmid, and ~40% of the termini retained at least 35 bp of non-telomeric DNA after telomere formation. It has been argued that the addition of $C_{1-3}A$ repeats to termini capped by non-telomeric DNA can not occur by recombination[4]. However, recombination reactions both *in vitro*[7] and *in vivo*[8] can bypass a heterology. It is therefore possible that telomere formation,

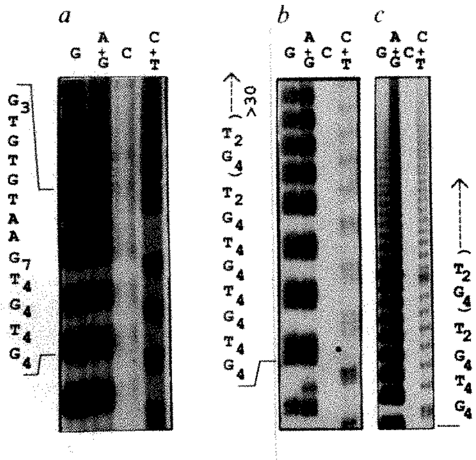

FIG. 2 DNA sequence of C_4A_4 termini. Sequences of C_4A_4 termini from plasmids derived from two independent yeast transformants are shown. *a*, Sequence of the junction between G_4T_4 repeats and the added $G_{1-3}T$ repeats in a plasmid that did not undergo recombination, YLp108CA-1. *b*, Sequence of the junction between G_4T_4 and G_4T_2 repeats in a plasmid that underwent recombination, YLp28CA-3. *c*, Long tract of G_4T_2 repeats (over 30 copies) are detected at the C_4A_4 end in clone YLp28CA-3.

METHODS. Telomere clones YLp108CA-1 and YLp28CA-3 were constructed as follows. Total DNA from yeast cells containing the linear plasmids YLp108CA or YLp28CA was digested with *Bgl*II or *Sph*I, respectively, treated with T4 DNA polymerase in the presence of dNTPs and self-ligated. Ligation mixtures were used to transform *E. coli* strain BSJ72 to ampicillin resistance. Telomere clones derived from YLp108CA were further digested with *Hin*dIII and *Sal*I and the fragments containing the telomeric sequences were subcloned into *Hin*dIII and *Sal*I digested plasmid pVZ1[25]. For each yeast transformant, telomere clones were isolated and sequenced from two independent *E. coli* transformants. In each case, the sequence at the junction between G_4T_4 repeats and the added $G_{1-3}T$ or G_4T_2 repeats was found to be identical. Sequencing analysis was conducted by the method of Maxam and Gilbert[22].

even on termini capped by a stretch of non-telomeric DNA, involves recombination, especially if the last few bases of the substrate are able to pair with $C_{1-3}A$ sequence. Alternatively, if telomerase[9-12] exists in *Saccharomyces* and if it can use a substrate in which the telomeric sequences are subterminal, it could mediate those telomere formation events that do not involve recombination.

Our results indicate that telomere formation in yeast is accompanied by telomere-telomere recombination. This recombination requires surprisingly little homology, perhaps because it is a site-specific event promoted by the ability of telomere DNA to assume non-B form structures involving triplex or quadruplex associations[13,14] or G-G[13-15] or C-C base pairing[16]. The junction between telomeric and unique sequence DNA, an apparent hot spot of recombination *in vivo*, also acts as a boundary for Klenow DNA polymerase and S1 nuclease *in vitro*[17,18]. Although the data here and elsewhere[4] do not rule out alternative pathways for telomere formation in yeast, telomere-telomere recombination provides an efficient mechanism for immediate rescue of DNA termini with very short stretches of telomere DNA. In contrast, other events that alter telomere length in yeast do so gradually such that many generations are needed to see a change in telomere length (reviewed in ref. 19). A working model for telomere formation in yeast by recombination is shown in Fig. 8. This scheme is reminiscent of the bacteriophage T4 'copy choice' model[8,20] that allows complete replication of the T4 genome by a process that requires recombination. As this recombination is non-reciprocal and can result in a net increase in telomere sequences it could contribute to telomere replication during the normal cell cycle.

If telomere-telomere recombination is important for telomere replication or maintenance, we also expect authentic telomeres to recombine. However, we did not detect transfer of C_4A_4 or C_4A_2 DNA to yeast chromosomes. These results can be explained if yeast telomeres are more likely to recombine with each other than with plasmid termini. This is possible as (1) chromosomal telomeres and internal stretches of $C_{1-3}A$ are more abundant than plasmid termini, and (2) chromosomal telomeres have much greater homology to each other than to C_4A_4 or C_4A_2 DNA. Alternatively, if telomere-telomere recombination is a salvage pathway that acts only on those telomeres with very short stretches of $C_{1-3}A$ DNA, its occurrence at natural chromosomes may be too rare to detect by Southern hybridization. □

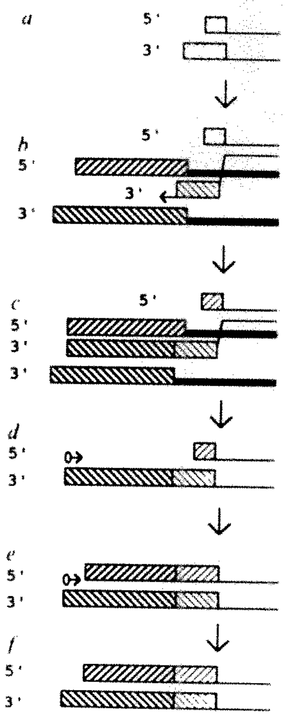

FIG. 3 Model for telomere formation by recombination. Different telomeric repeat sequences (thin-line hatched box) can promote telomere formation in yeast by serving as substrates for the addition of yeast $C_{1-3}A$ repeats (thick-line hatched box). In both cases, the C-rich strand is marked with 5′ and the G-rich strand is marked with 3′. Only one terminus of either a linear plasmid or a chromosome is shown. *a*, After replication of the plasmid in yeast and removal of the RNA primer, a single-strand tail is left at the 3′ end of the newly repliciated strand (represented by the unpaired thin-line hatched box). *b*, The 3′ OH end of the single-strand G-rich tail invades another telomere (donor, represented by thick-line hatched box). At the donor end, most (or all) recombination events are initiated (or resolved) at the junction between telomeric DNA (thick-lined hatched box) and unique DNA (filled-in bar). *c*, The invading G-rich strand is extended by replication using the donor telomere as a template. *d*, After dissociation, the terminus carries an ~300 nucleotide long $G_{1-3}T$ single-strand tail and serves as a template for primase (0) and conventional DNA polymerase mediated replication of the complementary strand (*e*). *f*, Subsequent removal of the RNA primer would still leave a gap at the 5′ end of the newly replicated strand, but no sequence information would be lost. (Alternatively, the extended G-rich strand could fold back on itself to form a terminal hairpin and provide the primer for replication of the C-rich strand[15].)

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1. Pluta, A. & Zakian, V. *Nature* **337,** 429–433 (1989).
2. Walmsley, R., Chan, C., Tye, B.-K. & Petes, T. *Nature* **310,** 157–160 (1984).
3. Szostak, J. W. *Nature* **337,** 303–304 (1989).
4. Murray, A., Claus, T. & Szostak, J. *Molec. cell. Biol.* **8,** 4642–4650 (1988).
5. Ahn, B.-Y., Dornfeld, K. J., Fagrelius, T. J. & Livingston, D. M. *Molec. cell. Biol.* **8,** 2442–2448 (1988).
6. Pluta, A., Dani, G., Spear, B. & Zakian, V. *Proc. natn. Acad. Sci. U.S.A.* **81,** 1475–1479 (1984).
7. Bianchi, M. E. & Radding, C. M. *Cell* **35,** 511–520 (1983).
8. Brunier, D., Michel, B. & Ehrlich, S. D. *Cell* **52,** 883–892 (1988).
9. Greider, C. W. & Blackburn, E. H. *Cell* **43,** 405–413 (1985).
10. Zahler, A. M. & Prescott, D. M. *Nucleic Acids Res.* **16,** 6953–6972 (1988).
11. Shippen-Lentz, D. & Blackburn, E. H. *Molec. cell. Biol.* **9,** 2761–2764 (1989).
12. Morin, G. *Cell* **59,** 521–529 (1989).
13. Sen, D. & Gilbert, W. *Nature* **334,** 364–366 (1988).
14. Williamson, J. R., Raghuraman, M. K. & Cech, T. R. *Cell* **59,** 871–880 (1989).
15. Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I. Jr & Blackburn, E. H. *Cell* **51,** 899–908 (1987).
16. Lyamichev, V. I. *et al. Nature* **339,** 634–637 (1989).
17. Henderson, E. *et al.* in *Eukaryotic DNA Replication* Vol. 6, (eds Stillman, B. & Kelley, T.) 453–461 (Cold Spring Harbor Laboratory, New York, 1988).
18. Budarf, M. & Blackburn, E. *Nucleic Acids Res.* **15,** 6273–6292 (1987).
19. Zakian, V. A. *A. Rev. Genet.* **23,** 579–604 (1989).
20. Mosig, G. *A. Rev. Genet.* **21,** 347–371 (1987).
21. Dunn, B., Szauter, P., Pardue, M. L. & Szostak, J. W. *Cell* **39,** 191–201 (1984).
22. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
23. Blackburn, E. H. & Gall, J. *J. molec. Biol.* **120,** 33–53 (1978).
24. Henderson, E. R. & Blackburn, E. H. *Molec. cell. Biol.* **9,** 345–348 (1989).
25. Henikoff, S. & Eghtedarzadeh, M. K. *Genetics* **117,** 711–725 (1985).

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Telomeres shorten during ageing of human fibroblasts

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THE terminus of a DNA helix has been called its Achilles' heel[1]. Thus to prevent possible incomplete replication[2] and instability[3,4] of the termini of linear DNA, eukaryotic chromosomes end in characteristic repetitive DNA sequences within specialized structures called telomeres[5]. In immortal cells, loss of telomeric DNA due to degradation or incomplete replication is apparently balanced by telomere elongation[6-10], which may involve *de novo* synthesis of additional repeats by a novel DNA polymerase called telomerase[11-14]. Such a polymerase has been recently detected in HeLa cells[15]. It has been proposed that the finite doubling capacity

TABLE 1 Effect of donor age on telomere length in human fibroblasts

Cell strain	Age *in vivo* years	 *in vitro* MPD (MPD max)	Mean telomere length kb ± s.d. (*n*)
HSC172	Fetal	18–28 (88)	8.6 ± 0.5 (3)
A30S	0	33 (58)	7.3 (1)
A38	24	31–33 (68)	6.9 ± 0.3 (2)
A35	70	19 (41)	6.7 (1)
F001	71	21–29 (40)	6.5 ± 0.4 (5)
F002	91	18–20 (45)	6.2 ± 0.1 (3)

Mean telomere length (the length of the terminal restriction fragment) was determined as described in Fig. 2*a* for fibroblast cell strains at the earliest available mean population doubling (MPD) in separate experiments. Strains were derived from female fetal lung (HSC172), female newborn skin (A30S), male forearm skin (A38, A35) or female abdominal skin (F001, F002). MPD at time of assay and senescence (MPD max) are indicated. The correlation between increasing donor age and decreasing telomere length is statistically significant ($P < 0.05$).

of normal mammalian cells is due to a loss of telomeric DNA and eventual deletion of essential sequences[1,16,17]. In yeast, the *est1* mutation causes gradual loss of telomeric DNA and eventual cell death mimicking senescence in higher eukaryotic cells[17]. Here, we show that the amount and length of telomeric DNA in human fibroblasts does in fact decrease as a function of serial passage during ageing *in vitro* and possibly *in vivo*. It is not known whether this loss of DNA has a causal role in senescence.

The finite replicative capacity of human fibroblasts in culture[18] is well established and has been extensively used as a model of cellular senescence[19]. To investigate the effect of cell division and age on telomeric DNA, we grew nontransformed human fibroblasts *in vitro* until terminal passage and regularly determined the size distribution of terminal restriction fragments (that is, telomeres) by Southern blot analysis (Fig. 1). Mean telomere length decreased about 2 kilobases (kb) with cumulative population doublings in five strains of fibroblasts studied (Fig. 2*a*). Moreover, the total amount of telomeric DNA also decreased (Fig. 2*b*), which indicates true loss of DNA and not simply rearrangement of telomeres with respect to restriction enzyme cleavage sites. Loss of telomeric DNA did not result from general degradation or loss of repetitive DNA in preparations of DNA from old cells[20], as other repetitive, non-telomeric sequence elements analysed in these experiments were not altered in size or amount (Fig. 1*b*, *d*). The loss was also not a function of growth state: cells at similar passage levels collected during exponential growth or during quiescence showed no significant difference in telomere distribution (not shown).

As loss of telomeric DNA observed with ageing *in vitro* may occur generally as somatic cells divide *in vivo*, it is important to determine the effect of donor age and tissue type on telomere length. Telomere length in fibroblasts varies between individuals and may become shorter during ageing *in vivo* (Figs 1 and 2, and Table 1). The increased heterogeneity of telomere lengths in cells from old donors (Fig. 1*c*, *g*) presumably reflects their variable history *in vivo* as well as their increased age in cell doublings. A larger population of individuals and other tissues must, however, be studied with both repetitive and unique-sequence telomere probes to confirm these observations. In addition, comparative studies should attempt to correlate telomere loss with cellular life span in different species.

The degree of telomere shortening observed during ageing of cultured human fibroblasts could be biologically significant. The ends of human telomeres consist of repeats of the sequence TTAGGG which are probably functionally important[21]. Human sperm telomeres have about 9 kb of TTAGGG repeats, whereas somatic cell telomeres may have only 4 kb. In addition, there seem to be variant forms of TTAGGG (such as TTGGGG) present sub-terminally[21]. The rates of loss of length and amount of telomeric DNA we have observed (Fig. 2) are in rough agreement with the estimate of 4 kb of TTAGGG per 'young' somatic telomere. Thus, the loss of 2 kb of terminal sequences could represent loss of most of the functional telomeric sequences. Even if there is substantially more than 4 kb of functional sequence at telomeres from young cells, this shortening

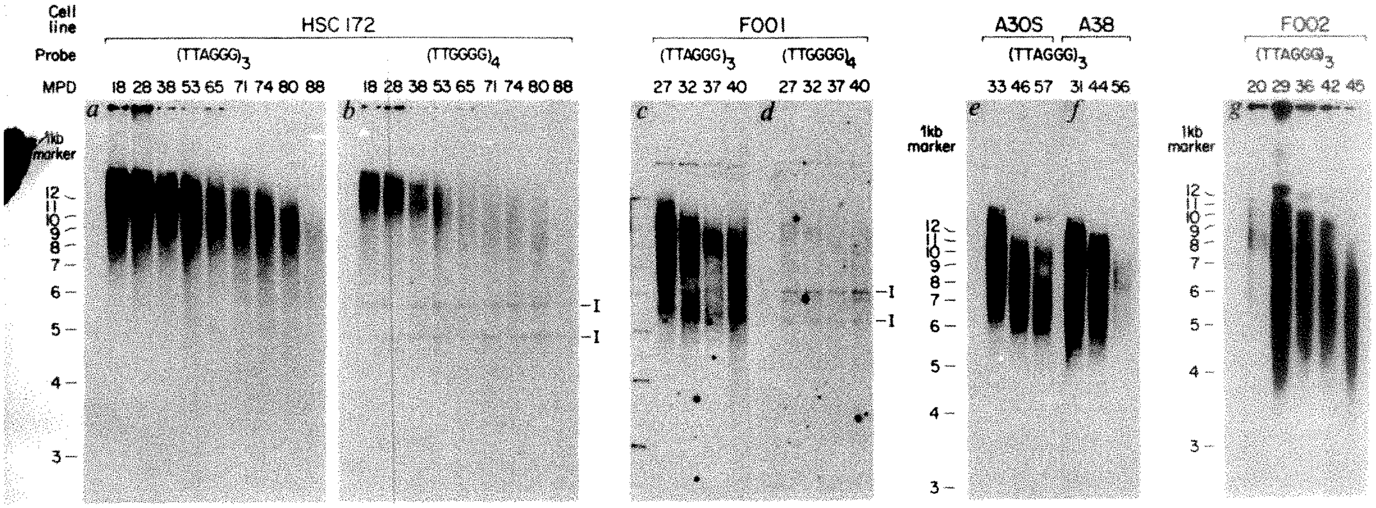

FIG. 1 Terminal restriction fragment (telomere) length in cultured human fibroblasts. Genomic DNA from fetal cell strain HSC172 (*a*, *b*), old adult F001 (*c*, *d*), F002 (*g*), newborn A30S (*e*) and young adult A38 (*f*) cell strains (see Table 1) was analysed at indicated mean population doublings (MPD) on Southern blots. Oligonucleotide probe $(TTAGGG)_3$ was used with high stringency washes to specifically detect telomeric sequences (*a*, *c*, *e*, *f*, *g*) and oligonucleotide $(TTGGGG)_4$ with low stringency washes to detect both telomeric sequences and internal repetitive sequences (I) (*b*, *d*). The size (kb) and position of markers are indicated.

METHODS. Cells were grown and population doublings counted as described[31] and DNA isolated when cells reached confluence. DNA was digested to completion with restriction enzymes *Msp*I and *Rsa*I and carefully quantified by a fluorometric method[32]. A portion (1–2 μg) was loaded onto a 0.7% agarose gel and resolved by electrophoresis at 1 V cm^{-1} for 36–72 h. For F002, only 1 μg was loaded in the first lane (MPD 20), 4 μg in the second lane (MPD 29) and 2 μg in each of the other lanes. DNA was depurinated by soaking gels in 0.1 M sodium citrate (pH 3.0) for 2 h, transferred to Nytran (Schleicher and Schuel) and hybridized in 6× SSC at 37 °C or 50 °C with end-labelled $(TTAGGG)_3$ or $(TTGGGG)_4$ respectively. Filters were washed in 3× SSC at 42 °C for the $(TTAGGG)_3$ probe or 4× SSC at 50 °C for the $(TTGGGG)_4$ probe.

could still be significant. As each cell contains 92 telomeres and the distribution of telomere lengths is wide, a loss of 2 kb from the mean length may imply a large increase in the proportion of cells missing TTAGGG from at least one telomere. The loss of even one telomere could cause the permanent cell-cycle arrest and chromosomal instability characteristic of senescent fibroblasts[22]. Most significantly, chromosomal abnormalities increase dramatically in the terminal phase of fibroblast cultures[23] and about 90% of these are dicentrics attached at their telomeres[24]. Loss of critical telomere sequences in late-passage fibroblasts could explain these data. Whether loss of telomeric DNA *in vivo* might contribute to the elevated (but still relatively low) frequency of chromosomal rearrangements, particularly dicentrics, observed in older humans[25,26] is uncertain.

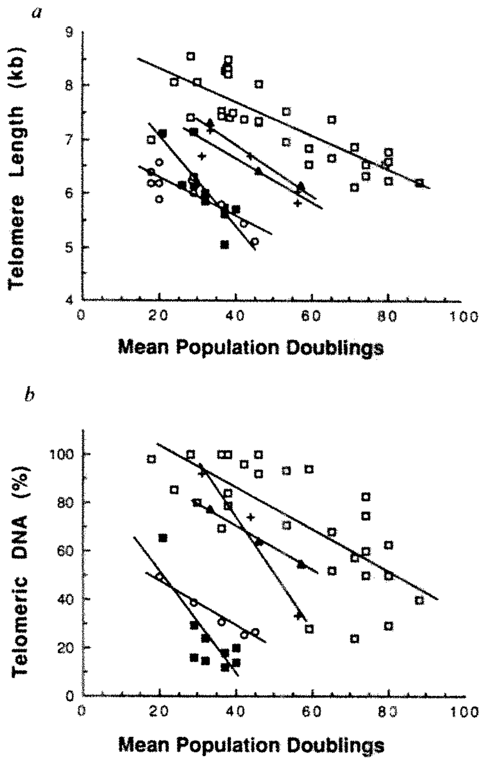

FIG. 2 Decrease in amount of telomeric DNA during replicative ageing of cultured human fibroblasts. Mean telomere length (*a*) and total telomeric DNA (*b*) are shown as a function of *in vitro* age. Symbols represent fetal cell strain HSC172 (□), newborn strain A30S (▲), young adult strain A38 (+), and old adult strains F001 (■) and F002 (○) (see Table 1). The slopes of the linear regression lines are: -31 bp MPD^{-1}, -0.9% MPD^{-1} (HSC172); -48 bp MPD^{-1}, -0.9% MPD^{-1} (A30S); -41 bp MPD^{-1}, -2.3% MPD^{-1} (A38); -85 bp MPD^{-1}, -2.1% MPD^{-1} (F001); and -37 bp MPD^{-1}, -1.0% MPD^{-1} (F002). Analysis of HSC172 cells include data collected from two separate ampules of cells grown at different times.

METHODS. DNA digested with *Msp*I and *Rsa*I was quantified and 2 μg run in each lane of various 0.7% agarose gels for transfer to Nytran filters and hybridization with $(TTAGGG)_3$ as described in Fig. 1. Autoradiographs were generated without an intensifying screen using the linear response range of the film and scanned with a densitometer. The signals probably include hybridization to variant forms of TTAGGG. Output was digitized. Mean telomere length was defined as $\Sigma(OD_i)/\Sigma(OD_i/L_i)$, where OD_i is the densitometer output (arbitrary units) and L_i is the length of the DNA at position i. Sums were calculated over the range 3–17 kb. This calculation assumes that DNA transfers at equal efficiency from all points in the gel and that the number of target sequences (telomere repeats) per DNA fragment is proportional to DNA length. Although neither of these assumptions is strictly true, the data did not warrant a more complicated model. Qualitatively similar results were obtained with other assumptions and methods. To estimate loss of total telomeric DNA, the integrated signal (ΣOD_i from 3–17 kb) was determined for each lane and, where possible, normalized to the signal from other Southern blots using a control probe. Lanes in which amounts other than 2 μg of DNA had been loaded were omitted from the analysis. Integrated signals for each autoradiograph were then expressed as a percentage of the signal from early passage HSC172 DNA on each autoradiograph as a standard.

Tumour cells are also characterized by shortened telomeres[27,33] and increased frequency of aneuploidy, including telomeric associations[28]. If loss of telomeric DNA ultimately causes cell-cycle arrest in normal cells, the final steps in this process may be blocked in immortalized cells. Whereas normal cells with relatively long telomeres and a senescent phenotype may contain little or no telomerase activity, tumour cells with short telomeres may have significant telomerase activity. Telomerase may therefore be an effective target for anti-tumour drugs.

In most unicellular eukaryotes, telomere length is a dynamic variable with both expansion and contraction events occurring under different culture conditions[5,7,8]. Complex regulation of telomere length is not unexpected in immortal, single-cell organisms which experience dramatic changes in their environment. However, it is possible that mortal, somatic cells of higher eukaryotes lack mechanisms for telomere elongation. Telomeric sequences may be added exclusively in the germ line, thus explaining the observation that sperm telomeres are longer than telomeres from normal somatic cells[17,21,29], and these telomeres may simply be lost during the life span of an individual.

There are a number of possible mechanisms for loss of telomeric DNA during ageing, including incomplete replication, degradation of termini (specific or nonspecific), and unequal recombination coupled to selection of cells with shorter telomeres[30]. Two features of our data are relevant to this question. First, the decrease in mean telomere length is about 50 bp per mean population doubling (Fig. 2*a*) and, second, the distribution does not change substantially with growth state or cell arrest. These data are most easily explained by incomplete copying of the template strands at their 3′ termini. But the absence of detailed information about the mode of replication or degree of recombination at telomeres means that none of these mechanisms can be ruled out. Further research is required to determine the mechanism of telomere shortening in human fibroblasts and its significance to cellular senescence. □

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1. Olovnikov, A. M. *J. theor. Biol.* **41,** 181–190 (1973).
2. Watson, J. D. *Nature new Biol.* **239,** 197–201 (1972).
3. Mueller, H. J. *Collecting Net* **13,** 181–198 (1938).
4. McClintock, B. *Genetics* **26,** 234–282 (1941).
5. Blackburn, E. H. & Szostak, J. W. *A. Rev. Biochem.* **53,** 163–194 (1984).
6. Bernards, A., Michels, P. A. M., Lincke, C. R. & Borst, P. *Nature* **303,** 592–597 (1983).
7. Shampay, J., Szostak, J. W. & Blackburn, E. H. *Nature* **310,** 154–157 (1984).
8. Larson, D. D., Spangler, E. A. & Blackburn, E. H. *Cell* **50,** 477–483 (1987).
9. Shampay, J. & Blackburn, E. H. *Proc. natn. Acad. Sci. U.S.A.* **85,** 534–538 (1988).
10. Murray, A. W., Claus, T. E. & Szostak, J. W. *Molec. cell. Biol.* **8,** 4642–4650 (1988).
11. Greider, C. W. & Blackburn, E. H. *Nature* **337,** 331–337 (1989).
12. Greider, C. W. & Blackburn, E. H. *Cell* **43,** 405–413 (1985).
13. Zahler, A. M. & Prescott, D. M. *Nucleic Acids Res.* **16,** 6953–6972 (1988).
14. Shippen-Lentz, D. & Blackburn, E. H. *Molec. cell Biol.* **9,** 2761–2764 (1989).
15. Morin, G. B. *Cell* **59,** 521–529 (1989).
16. Cooke, H. J. & Smith, B. A. *Cold Spring Harb. Symp. quant. Biol.* **51,** 213–219 (1986).
17. Lundblad, V. & Szostak, J. W. *Cell* **57,** 633–643 (1989).
18. Hayflick, L. & Moorhead, P. S. *Expl Cell Res.* **25,** 585–621 (1961).
19. Stanulis-Praeger, B. M. *Mech. Ag. Dev.* **38,** 1–48 (1987).
20. Prashad, N. & Cutler, R. G. *Biochim. biophys. Acta* **418,** 1–23 (1976).
21. Allshire, R. C., Dempster, M. & Hastie, N. D. *Nucleic Acids Res.* **17,** 4611–4627 (1989).
22. Saksela, E. & Moorhead, P. S. *Proc. natn. Acad. Sci. U.S.A.* **50,** 390–395 (1963).
23. Sherwood, S. W., Rush, D., Ellsworth, J. L. & Schimke, R. T. *Proc. natn Acad. Sci. U.S.A.* **85,** 9086–9090 (1989).
24. Benn, P. A. *Am. J. hum. Genet.* **28,** 465–473 (1976).
25. Prieur, M. *et al. Hum. Genet.* **79,** 147–150 (1988).
26. Bender, M. A., Preston, R. J., Leonard, R. C., Pyatt, B. E. & Gooch, P. C. *Mutat. Res.* **212,** 149–154 (1989).
27. Hastie, N. D. & Allshire, R. C. *Trends Genet.* **5,** 326–331 (1989).
28. Pathak, S., Wang, Z., Dhaliway, M. K. & Sacks, P. C. *Cytogenet. Cell Genet.* **47,** 227–229 (1988).
29. Cross, S. H., Allshire, R. C., McKay, S., McGill, N. & Cooke, H. J. *Nature* **338,** 771–774 (1989).
30. Harley, C. B., Shmookler Reis, R. J. & Goldstein, S. *J. theor. Biol.* **94,** 1–12 (1982).
31. Harley, C. B. *Meth. molec. Biol.* **5,** 25–32 (1990).
32. Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L. & Evans, D. H. *Nucleic Acids Res.* **7,** 547–569 (1979).
33. de Lange, T. *et al. Molec. cell. Biol.* **10,** 518–527 (1990).

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(3761)A

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(W7382)A

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The University is an Equal Opportunity Employer. (3739)A

Head — Department of Agronomy

Nominations and applications are invited for the position of Department Head and Professor of Agronomy at Purdue University. The department head provides leadership while administering research, teaching, extension, and international programs of the Department of Agronomy. Candidates must have a Ph.D. degree, be eligible for tenure at the rank of full professor, and have professional experience in an area related to departmental programs. The department head should have leadership and administrative abilities, appreciation for the diverse interests and missions of the department, and support the philosophy of the Land Grant university. Applicants should send a letter of application; a statement of philosophy emphasizing their ability to foster faculty development and program growth into the 21st century; curriculum vitae, and names, addresses, and telephone numbers of five references. Applications or nominations must be received by August 1, 1990 (or until a suitable candidate is found). Send applications to: **Dr. R.L. Thompson, Dean of Agriculture, ATTN: Agronomy Head Search Committee, Agricultural Administration Building, Purdue University, West Lafayette, Indiana 47907-7899.** *Purdue University is an Equal Opportunity/Affirmative Action Employer.* Women and minorities are encouraged to apply.

(NW5372)A

UNIVERSITY OF LIVERPOOL
REGIONAL CYTOGENETICS UNIT
CYTOGENETICS UNIT

SENIOR SCIENTIFIC OFFICER

Salary scales are in accordance with Whitley Council £12,644-£16,639 p.a. (pending grading)

The Unit provides a comprehensive cytogenetics service for the Mersey Regional Health Authority.

Applicants should have at least four years' experience in cytogenetics and a particular interest in prenatal diagnosis would also be beneficial.

Quote ref: RV/707/NAT
Closing date: 18 June, 1990

Applications, by c.v. with the names of three referees, should be received by the **Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX,** from whom further particulars may be obtained.

An Equal Opportunity Employer (3731)A

UNIVERSITY OF SUSSEX
School of Biological Sciences

Post-Doctoral Research Fellow

Applications are invited for a Post-Doctoral Research Fellow to participate in a project to make a biochemical dissection of a cell-free system from Xenopus eggs, which is capable of efficient DNA synthesis and specifically of DNA end joining and associated recombination and repair reactions. Applicants should preferably, have experience in protein biochemistry, DNA enzymology and molecular biology.

Salary within Grade 1A of the Research and Analogous Faculty Salary scale £10,458 to £16,665 per annum.

Further information can be obtained from, and applications should be submitted to **Professor Sydney Shall and Dr. Chris Ford,** together with the names of two referees, **School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG**. Telephone, Professor Shall, (0273) 678303.

AN EQUAL OPPORTUNITY EMPLOYER (3734)A

Ph.D. Student in Molecular Biology at the University of Basel

At the lab of molecular endocrinology our main research topic is the regulation of IGF (insulin-like growth factor) activity at the molecular and cellular level. The work will consist of "classic" molecular biology and its application in different cellular systems. The position can be filled as of July 1, 1990. Applications with full CV and the names of two referees should be sent to:

Dr. Jürg Schwander
Department of Internal Medicine
Kantonsspital
4031 Basel Switzerland
Telefax (41) 61 35 64 33

(W7376)A

Cancer Research Campaign

- We are the biggest funder of cancer research scientists, supporting some 700 projects in universities, medical schools and research institutes throughout Britain.
- We have developed a strong interface between research and clinical therapy, facilitating the effective development and implementation of cancer treatments.
- Of the nearly £40 million we raised through voluntary contributions in 1989, 92% went directly into research - accounting for around 40% of all cancer research in the UK.

THE BEATSON INSTITUTE FOR CANCER RESEARCH

The Beatson Institute for Cancer Research, Garscube Estate, Bearsden Road, Glasgow, G61 1BD. Tel. 041-942 9361. Fax 041-942 6521.This is a modern, well-equipped laboratory with 10 groups working in various areas of gene expression and tumour biology.

POST-DOCTORAL SCIENTIST

A new vacancy has arisen for a Post-Doctoral Scientist with interests in molecular characterisation of growth factors and their receptors involved in the control of stem cell proliferation in normal and neoplastic tissue (see Nature 344, 442, 1990). Applicants should have a Ph.D. and relevant experience in molecular biology. Salary is in the range £13,527 to £16,665 per annum and is dependent on age and experience. Applications with C.V. and names of two referees should be sent to Dr. I. B. Pragnell at the Beatson Institute for Cancer Research.

SCIENTIFIC OFFICER

A vacancy has arisen for a Scientific Officer to work in a group with interests in the control of stem cell proliferation in normal and neoplastic tissue. Applicants should have an Honours degree or equivalent and a strong interest in cellular biology. A background in haematology would be advantageous. Starting salary is in the range £9,679 to £12,956 per annum. Applications with C.V. and names of two referees should be sent to Dr. I. B. Pragnell at the Beatson Institute for Cancer Research.

SCIENTIFIC OFFICER

A new position has become available for a Scientific Officer to work with a Research Group studying the control of stem cell proliferation in normal and neoplastic tissue. Applicants should have an Honours degree or equivalent, and relevant experience in the application of molecular biology techniques to biological problems. Starting salary is in the range £9,679 to £12,956 per annum.

Applications with C.V. and names of two referees should be sent to Dr. I. B. Pragnell at the Beatson Institute for Cancer Research.

PATERSON INSTITUTE FOR CANCER RESEARCH

Christie Hospital & Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX. Tel: 061-445 8123 Ext. 557; Fax: 061-434 7728.

POST-DOCTORAL RESEARCH FELLOWSHIP

Department of Molecular Biology

A post-doctoral research fellowship is available, starting 1 August 1990. The initial appointment will be for a period of three years. Salary will be in the range £12,381 - £15,371 p.a. (under review).

The work of the Department is centred around Epstein-Barr Virus and its interaction with epithelial cells and involvement in nasopharyngeal carcinoma. Current interests include expression and transfection of EBV genes, structural and immunological studies, vaccine development and transformation of epithelial cells.

Applications are invited from individuals with interest and/or expertise in any area compatible with this framework of research.

Applications, giving full CV and names of 2 academic referees, or informal enquiries, to Dr. J. R. Arrand, Paterson Institute for Cancer Research. Closing date 21 June 1990. (3767)A

Fighting cancer on all fronts.

POSTDOCTORAL RESEARCH ASSOCIATE
BROOKHAVEN NATIONAL LABORATORY

The Biology Department has a position available for a recent Ph.D. experienced in (bio)chemical separation methods, including HPLC, gel electrophoresis, thin layer chromatography and some experience with enzymology. Knowledge of x-ray/neutron diffraction methods is desirable. Research would include enzymatic synthesis of messenger RNA analogues used to generate and purify tRNA-ribosome and initiation factor-ribosome complexes used in diffraction studies designed to determine the physical location of the corresponding ribosomal binding sites.

Applicants should submit curriculum vitae with a list of publications, statement of research interests and names of three references to: **Dr. Malcolm S. Capel, Biology Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, NY 11973.** EOE M/F.

(NW5352)A

MOLECULAR BIOLOGY POSTDOCTORAL RESEARCH ASSOCIATE(S)

needed to study the function of identified genes that code enzymes that release oligosaccharins. One project concerns genes involved in host-pathogen interactions; the other concerns genes involved in the regulation of flower formation. Ph.D. degree required. Individual(s) selected will have experience in molecular biology. Salary $18,000-24,000 depending on experience. Send applications (to be evaluated beginning July 1, 1990, with a hiring decision made soon thereafter) to **Dr. Alan Darvill or Dr. Peter Albersheim, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602.** *An AA/EEO institution.* (NW5357)A

UNIVERSITY COLLEGE LONDON
WITCHWEED RESEARCH AT UCL

UCL

Applications are invited for the position of

Postdoctoral Research Associate

in the **STRIGA RESEARCH GROUP.**

The main investigation will be the study of haustorial development and function. Experience in molecular cell biology is preferred although a good biochemical background would be acceptable. The appointment is funded by the SERC and is available for up to three years.

Salary will be on RA1A scale (£12,855-£14,148).

Applications to be sent to **Professor G. R. Stewart, Department of Biology, University College London, Gower Street, London WC1E 6BT.** Telephone No. 071 387 7050 ext. 2653. *Equal Opportunities Employer.* (3715)A

MRC Human Biochemical Genetics Unit and St Mary's Hospital Chest Clinic

SCIENTIFIC OFFICER

(MRC Scales)

required for a collaborative project to work on the molecular genetics of mucin polymorphisms in relation to susceptibility to lung disease (funded by the CHEST, HEART & STOKE Association). This job would suit a new graduate, and would include some contact with patients and working with clinicians as well as scientists. Enquiries and applications to **Dr. D.M. Swallow, MRC H.B.G.U., University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE,** Tel: 071-387 7050 Ext. 5040.

(3740)A

LEUKAEMIA RESEARCH FUND CENTRE
at the INSTITUTE OF CANCER RESEARCH

Patron of the Fund:
HRH The Duke of Kent

Team Leader

Fulham Road, London SW3

Salary up to £28,000

The Institute of Cancer Research is one of the world's leading cancer research centres. The Leukaemia Research Centre (Director: Professor M. F. Greaves) has a major research programme on the cellular and molecular biology of leukaemia and normal haemopoietic differentiation.

We are seeking a Senior Scientist with at least five years post-doctoral experience in molecular genetics or related fields to initiate projects complementary to the existing interests of the LRF Centre.

The post is for a three year (or possibly five year) period in the first instance.

Salary is likely to be in the range of £19,105 to £23,961, or £25,073 to £28,121 inclusive, according to experience.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

C.V.'s, in duplicate, with the names and addresses of two referees should be sent to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference number 6.90.S.N.11.

(3768)A

UNIVERSITY OF GLASGO
INSTITUTE OF PHYSIOLOGY
POST-DOCTORAL RESEARCH ASSISTAN

Applications are invite from candidates with background in electrophysi ology to carry out a proje to study the excitability isolated nerve fibres.

The project will be a pilo study and the appointmen is for 1 year in the first instance. The salary is £11,39 per annum (subject to review). The post will be available from 1st Septembe 1990.

Enquiries or applications by letter, incuding C.V. (2 copies) and names and addresses of 2 academic referees, should be sent by 20th June 1990 to **Dr R. Orchardson, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ (041 339 8855 Ext 6061).** (3716)A

UNIVERSITY OF NATAL

PIETERMARITZBURG,
SOUTH AFRICA

The University of Natal rejects apartheid. It is an equal opportunity, affirmative action university.

Department of Physics

Lecturer

Ref: P55/90
Closing date: 30 September 1990

The successful applicant will teach courses from beginning Physics to Honours Level, and contribute to research, preferably in the field of modern optics.

Applicants should have a Ph.D and preferably some teaching experience.

Leave Substitute Lecturer

Ref: P56/90
Closing date: 30 September 1990

This is a temporary post from February to November 1991. The incumbent will teach existing beginning and second year Physics courses.

Post Doctoral Associate

Ref: P57/90
Closing date: 31 July 1990

This is a temporary post to the end of 1991. The successful candidate will work on problems of metal fatigue with Professor P J Jackson, or initiate work in modern optics.

The salaries offered will be determined according to the qualifications and /or experience of the successful applicants.

Application forms, details of the salary package and conditions of service are obtainable from the Personnel Section, University of Natal, P O Box 375, Pietermaritzburg 3200, South Africa, telephone (0331) 63320, fax no: (0331) 63497 OR The Secretary, South African Universities Office, Second Floor, 16 Charles 11 Street, LONDON. Telephone; (071) 839-3420; fax no: 839-3480. (W7385)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
and
BRISTOL UNIVERSITY
DEPARTMENT OF BIOCHEMISTRY

A collaborative programme has been established with support of the European Community Laboratory Twinning scheme under the terms of which two scientific positions are now available for a period of up to three years to non-UK EC nationals for employment at the above member state institutions.

1. **National Institute for Medical Research, London (Dr D R Trentham and Dr M R Webb)**
   A postdoctoral scientist is required for work on the characterisation of processes associated with phosphate release during muscle contraction. The project will involve site-directed mutagenesis of a phosphate binding protein and its use as a probe of phosphate release. Techniques will also include protein expression and purification, protein labelling as well as muscle mechanics. Experience in some of these would be an advantage. The salary will be paid on the salary scale £12,381 to £15,372 (starting salary dependent on experience) plus £1,650 per annum London Weighting. MRC pension scheme option.
   Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, not later than 5/7/90 quoting reference number PPB/EC/PD. Informal enquiries may be made to Dr Trentham or Dr Webb on 081 959 3666 ext 2078: fax 081 959 4477.
2. **Bristol University, Department of Biochemistry (Professor H Gutfreund and Dr M Geeves)**
   A postdoctoral scientist is required to work on a study of the dynamic aspects of protein-protein and protein-nucleotide interactions in both solution and skinned muscle fibres. The work will involve attaching spectroscopic probes to both native and genetically modified muscle proteins. Experience of protein chemistry or transient kinetic methods would be an advantage but is not essential. The salary will be paid o[illegible] the scale £10,458 to £12,381 per annum.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Dr M Geeves, Department of Biochemistry, University of Bristol, Bristol, BS8 1TD. Tel. 0272 303030; fax 0272 303497** not later than 5/7/90.

The MRC and Bristol University are both Equal Opportunity

SCHERING

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Industrie-Chemikalien · Galvanotechnik

We are a leading chemical-pharmaceutical company in West-Germany with 25,000 employees worldwide. At our headquarters in Berlin we employ 7,000 people. Our pharmaceutical branch is committed to the development of new therapeutic agents through basic research.

For an interdisciplinary project on "Sexual Hormones and Hemostasis" we are looking for a young scientist for a two-year period (extension for a further year is possible) as a

Post-Doc: M. D. or PH. D.

for our department of Experimental Gynecology and Fertility Control.

Your task	☐ development of relevant animal models of thrombosis ☐ participation in experimental animal studies including statistical evaluation and documentation of obtained data
Your qualification	☐ M. D. with a biological background or PH. D. ☐ experience in hemostasis research ☐ familiarity with experimental animal models desirable
We offer you	an innovative task in close collaboration with a team of hemostaseologists, molecular biologists and gynecologists.
Berlin offers you	an outstanding cultural and educational environment.

Please forward your c.v., a list of publications, and a summary of your former research activities marked "confidential" to:

Schering Aktiengesellschaft
Personalabteilung FO
Müllerstr. 170-178, D-1000 Berlin 65

(W7378)A

UNIVERSITÄT BREMEN

Im Fachbereich 2 — Studiengang Biologie
— ist zum nächstmöglichen Zeitpunkt eine

Professur

Beamtin/Beamter auf Lebenszeit — Bes.Gr. C 3 —

mit dem Aufgabengebiet

"Mikrobiologie mit dem Schwerpunkt Marine Mikrobiologie"

Kennziffer: P 419/90

zu besetzen.

Der/Die Stelleninhaber/in vertritt das Fach Mikrobiologie in der Lehre des Diplom- und Lehramtsstudienganges Biologie und für Nebenfachstudentinnen und -studenten. An der Forschung beteiligt er/sie sich insbesondere am Sonderforschungsbereich 261 der DFG "Der Südatlantik im Spätquartär: Rekonstruktion von Stoffhaushalt und Stromsystemen".

Einstellungsvoraussetzungen u.a.:

— abgeschlossenes Hochschulstudium und Promotion
— hervorragende wissenschaftliche Leistungen auf dem Gebiet der Meeres- und Sedimentmikrobiologie
— Habilitation oder gleichwertige Qualifikation
— Lehrerfahrungen und pädagogische Eignung.

Die Universität Bremen strebt eine Erhöhung des Anteils von Frauen am wissenschaftlichen Personal an und fordert deshalb qualifizierte Wissenschaftlerinnen nachdrücklich auf, sich zu bewerben.

Interessenten werden gebeten, Informationsmaterial beim Fachbereich anzufordern. Bewerbungen sind unter Angaben der Kennziffer bis zum 30.6.90 zu richten an die

UNIVERSITÄT BREMEN
— Fachbereich 2 —
Leobener Straße/NW 2
D-2800 Bremen 33

(W7386)A

RESEARCH SCIENTIST MOLECULAR BIOLOGY

Amgen, an innovative leader in the discovery, development, and marketing of human therapeutics through applied biotechnology, has an immediate opportunity for a Research Scientist in our Molecular Biology Group.

Responsibilities for this position will include the development of research programs leading to the identification and cloning of novel cellular growth factors involved in stem cell regeneration and recruitment.

Qualified applicants must have a Ph.D. in Molecular or Cellular Biology and a minimum of 2 years' postdoctoral experience in the characterization of cellular growth factors, cDNA cloning, and the expression of recombinant proteins.

Amgen offers an excellent compensation and benefits package as well as a challenging research environment on the leading edge of biotechnology. If you have the necessary skills and qualifications and would like to be a part of an organization that places a high priority on its human resources, please send a complete CV, in confidence, to:

AMGEN, INC.
Recruitment, Dept. #527
Amgen Center
Thousand Oaks
CA 91320-1789

(NW5365)A

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Employer

AMGEN

RESEARCH CENTRE JÜLICH

We are a national research centre jointly financed by the Federal Republic of Germany and the Federal State of North Rhine-Westphalia with a staff of 4,500. The four major research priorities of environmental research, materials research, information technology and energy technology are part of a broad spectrum of basic research ranging from medicine and biotechnology to solid-state research and basic nuclear research.

For the INSTITUTE OF CHEMISTRY we are looking for an experienced scientist in the area of

HYDROGEOLOGY/ NUMERICAL SIMULATION

Job description: Investigation of transport problems and methods of simulation in inhomogenous, discontinuous porous media, such as sedimentary rocks and sediments. Especially important are simulation techniques for flow- and transport models, including coupled chemical reaction systems, which can handle anisotropies, inhomogeneities, and discontinuities of transport coefficients. These tasks will be an integral part of the QUANTITATIVE GEOLOGY GROUP whose objective is the construction of a workstation system to model geological processes in sedimentary basins. The goal is to predict the distribution of porosity, permeability and mobile substances, based on modelling the spatial and temporal interaction of mass and heat transfer and chemical reactions. Applications include oil and gas exploration and groundwater systems with environmental disposal problems.

Qualifications: Completed Ph.D. and excellent background and experience in applied mathematics including knowledge of finite element methods. Experience in the area of hydrodynamics/geohydrology or petroleum geology is highly desirable. Knowledge of physics or chemistry is necessary as well as the capability to design computer programs. In addition, the candidate should be able to adapt to the specifically geologic aspects of the work.

Please address inquiries and applications with C.V. to:

KFA

RESEARCH CENTRE JÜLICH
PVA-PE, P.O. Box 1913, 5170 Jülich
Fed. Rep. Germany
(Ph.: 49-2461/615358) (W7383)A

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF PHYSICAL BIOCHEMISTRY

POSTDOCTORAL SCIENTIST

Applications are invited for a three-year post-doctoral position funded by a grant from the Human Frontier Science Program to Dr D R Trentham, FRS for research into the mechanism of low molecular weight guanine nucleotide binding proteins, particularly those involved in signal transduction and smooth muscle regulation. Experience in protein purification, enzymology, fluorescence spectroscopy or molecular biology would be an advantage.

The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA**, not later than 29th June 1990, quoting reference number **PPB/HFSP**.

POST-GRADUATE STUDENTSHIP

An MRC postgraduate studentship is available in the same field. Application forms can be obtained from **Dr R W King, Director of Studies**, at the above address.

Informal enquiries about both positions can be made to Dr J F Eccleston on 081-959 3666 ext. 2081.

MRC
Medical Research Council

The MRC is an Equal Opportunities Employer. (3749)A

UNIVERSITY OF KEELE
Staffordshire

SCHOOL OF POSTGRADUATE-MEDICINE AND BIOLOGICAL SCIENCES

LECTURESHIP IN THE DEPARTMENTS OF MEDICINE AND BIOLOGICAL SCIENCES

This newly created post offers an exciting opportunity for an energetic non-clinical scientist to help build a research team applying state-of-the-art molecular biochemical techniques to investigating the pathogenetic mechanisms of human infertility and endocrine diseases. The post, funded jointly by the North Staffordshire Health Authority and Keele University, is primarily a research appointment with a limited teaching component. Experience in molecular biology/ molecular genetics would be an advantage.

Potential applicants seeking further information or wishing to visit the Departments should contact Dr. R.N. Clayton, Senior Lecturer in Medicine on (0782) 46100, extension 4295, or Professor J.B. Lloyd, Professor of Biochemistry on (0782) 621111, extension 3088. Salary on the scales £10,458 – £20,469 p.a. (under review).

Further details and application forms from the **Registrar, University of Keele, Keele, Staffs, ST5 5BG,** (Tel: (0782) 621111, extension 3008). Closing date: 22 June 1990. (3726)A

THE UNIVERSITY OF BIRMINGHAM SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH ASSOCIATE

This AFRC funded post is available for three years to investigate gene regulation of sarcomeric muscle proteins during the growth of the European lobster. The research will involve isolation of cDNA clones for crustacean muscle proteins. These clones will then be used to investigate the changes in gene transcription through the moult cycle. Graduates in biochemistry, genetics or animal biology are welcome to apply. Some theoretical knowledge of molecular biology would be an advantage. The successful applicant may be eligible to register for a higher degree.

Informal enquiries to Dr Alicia El Haj (021 414 5459).

Salary on a range fronm £9,816-£12,381 (currently under review).

Application forms (returnable by 20 June 1990) and further particulars available from **The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Telephone 021 414 6483** (24 hours). Quote ref: S13056.

The University is an equal opportunities employer. (3733)A

FACULTY POSITION/ TOXICOLOGIST

TOXICOLOGIST. An Assistant/Associate Professor, 12 month, tenure track, 60% extension and 40% research toxicology position is available in the Department of Food Science, Purdue University. Candidate should have a Ph.D., experience and strong interest in developing a toxicology program for the State of Indiana. Salary commensurate with experience. July 15, 1990 is the closing date or until qualified candidate is identified. Please send a letter of application, curriculum vitae, three letters of reference to: **Dr. P. E. Nelson, Head, Dept. of Food Science, Smith Hall, Purdue University, West Lafayette, IN 47907.** *Purdue is an Equal Opportunity/Affirmative Action Employer.* (NW5379)A

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British Geological Survey

Analytical Chemists

The British Geological Survey has vacancies for two chemists to work in the new laboratories of its Analytical Geochemistry Group based at Keyworth, near Nottingham.

Post 1

The appointee will be involved in providing analytical support for a wide range of geological and geochemical projects in the UK and overseas. This will involve the provision of analytical advice and carrying out analysis of rocks, soils and water for major and trace elements and organic constituents by appropriate techniques, including XRF and ICP spectrometry, GC and HPLC. Some research and development of analytical methods will also be involved.

Post 2

The appointee will be responsible for the day-to-day organisation of the ICP-AES laboratory, including the operation of a new computer-controlled simultaneous/sequential spectrometer for the analysis of a wide variety of geological samples; the implementation of a Quality Assurance scheme; and assisting with the development of new and improved analytical methodologies.

The appointments will be for a period of five years with the possibility of conversion to a permanent position.

Applicants should have a good honours degree in chemistry or other appropriate subject.

Salary will be within the range £10,678 to £14,909 for Post 1 and £9,131 to £12,223 for Post 2, depending on qualifications and experience. Terms include a generous leave allowance and a non-contributory pension scheme.

For an application form, to be returned no later than 14th June 1990, write to:- Establishments (Recruitment), British Geological Survey, Keyworth, Nottingham NG12 5GG. Please quote reference HSO/KW/90/2 and indicate for which post you are applying.

The British Geological Survey is a component Institute of the Natural Environment Research Council.

The Natural Environment Reseach Council is an equal opportunities employer.

**Natural
Environment
Research
Council**

(3760)A

GUY'S HOSPITAL
PURINE RESEARCH LABORATORY
LABORATORY ASSISTANT/TRAINEE MLSO

£6,710 - £8,071 per annum

We require the above technical post which will demand a high degree of accuracy and care in preparation of solutions for HPLC and the cleaning of glassware.

You would also be required to undertake TLC Plate-making and assist in chromatography and electrophoresis.

Experience, although desired, is not essential as full training will be given.

For application papers, please contact **Personnel Department, 1st Floor, Guy's Hospital, St. Thomas Street, London SE1 9RT.** Telephone number 071-955 5000 ext 5092 quoting ref. no. P/58.

Aiming to be an Equal Opportunities Employer. (3729)A

MOLECULAR VIROLOGIST
YALE UNIVERSITY SCHOOL OF MEDICINE

The Department of Epidemiology and Public Health invites applications for a position at the Assistant Professor level in the Yale Arbovirus Research Unit, available 1 January 1991. Candidates should have postdoctoral research experience in molecular virology, potential to obtain research support, and an interest in the molecular epidemiology, antigenic relationships, and/or evolution of arboviruses. The appointee will be expected to interact with other members of the Unit, to assist in arbovirus reference center activities, to develop an independent research program and to participate in teaching of graduate students. Send curriculum vitae, a summary of research interests and names of three references by 1 September 1990 to: **Dr. Robert E. Shope, Department of Epidemiology and Public Health, Yale University School of Medicine, P.O. Box 3333, New Haven, Connecticut 06510.** *Yale University is an Equal Opportunity/Affirmative Action Employer.* (NW5375)A

RESEARCH SCIENTIST

(Re-advertised)

$A32,471-$A47,070

DIVISION OF WOOL TECHNOLOGY
RYDE, NSW, AUSTRALIA

Are you a

- PHYSICIST
- TEXTILE SCIENTIST
- MATERIALS SCIENTIST
- ENGINEER

We need scientists who are innovative, lateral thinkers, capable of elegant solutions to complex problems, excited by new ideas, interested in computer service and sensor technology, not afraid of making mistakes and can spark ideas in others, then

HERE IS THE OPPORTUNITY FOR YOU

We urgently need your inventive skills to provide a range of solutions and opportunities to help Australia and wool compete on the world market.

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Training will be provided if required.

QUALIFICATIONS: PhD or equivalent.

TENURE: Indefinite Appointment with Australian Government Superannuation Benefits.

SALARY LEVEL: Research Scientist/Senior Research Scientist.

APPLICATIONS: Further technical details and a detailed job description and selection criteria may be obtained from Dr S. de Jong on (02) 800 211. Applications must state relevant personal details, details of qualifications and experience, the names of at least two referees and the reference number C89902.

Applications should reach:

**The Chief,
CSIRO, Division of Wool Technology,
P.O. Box 7, Ryde NSW 2112, Australia**
by 29th June, 1990.

CSIRO
AUSTRALIA

CSIRO PROMOTES A SMOKE-FREE ENVIRONMENT
CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W7380)A

UNIVERSITY OF DURHAM
LECTURESHIPS IN ANTHROPOLOGY

Two Lectureships in Anthropology are offered from 1st October 1990, or as soon as possible thereafter. One post will be a Lectureship in Biological Anthropology, the other a Lectureship in Social Anthropology.

The appointments will be made on the Lecturer Grade A scale £10,458 – £15,372 p.a. (currently under review).

Applications for the Lectureship in Social Anthropology are invited from candidates with research interests in any of the regional and theoretical areas of the discipline.

Applications for the Lectureship in Biological Anthropology are invited from candidates with research interests in any areas of the discipline as broadly defined.

The successful candidates will be expected to show commitment to the aims of a broadly based Anthropology Department, to teach core areas of their discipline outside their immediate research specialism, and to contribute to selected interdisciplinary aspects.

Further particulars may be obtained from **The Personnel Officer, Old Shire Hall, Durham DH1 3HP** (tel 091 374 4687) to whom applications (three copies, including a full CV and the names of three referees) should be sent not later than 20th June 1990. Please quote reference 537. (3753)A

LEICESTER UNIVERSITY
Department of Geology
EXPERIMENTAL OFFICER IN GEOPHYSICS

Applications are invited for a post of Experimental Officer (Grade 1) in Geophysics in the Department of Geology. The post is temporary for three years.

Applicants, preferably, should have knowledge of seismic and electrical/electromagnetic methods of geophysical surveying and be familiar with mainframe computing and the use of IBM PCs. The post would suit a graduate in Geophysics, or Physics or Geology. The main responsibility will be to manage, maintain and develop field data acquisition systems and the subsequent analysis of data in explosion and earthquake seismology and in Transient Electro-Magnetic methods. The department has a long record of projects in both methods which are in progress in the UK, Europe and Kenya. The post will involve a fair amount of fieldwork. A clean driving licence is essential.

Salary on the Other Related Grade 1 scale, £11,399 to £13,495 pa, depending on qualifications and experience.

Further particulars and an application form are available from the **Staffing Office (Ref EO/GPH), University of Leicester, University of Leicester, University Road, Leicester LE1 7RH, telephone (0533) 522438.**

Closing date for applications: 6 July 1990. (3754)A

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE
Senior Technician

To join an active research team in the Department of Anatomy, using a wide range of molecular biological techniques. Applicants should have some experience in the techniques and be able to contribute to this new and expanding research initiative. The position is not limited to the length of a specific grant and provides an ideal opportunity for career development.

Salary £8,904-£10,632.

Application forms (returnable by 21 June 1990) and further particulars available from the **Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT.** Telephone 021 414 6483 (24 hours). Quote ref M6751.

The University is an equal opportunities employer. (3752)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF OPHTHALMOLOGY AND MOLECULAR AND CELL BIOLOGY
POSTDOCTORAL RESEARCH FELLOW

Applications are invited from Molecular Biologists, Cell Biologists or persons qualified in other appropriate disciplines for a position as Postdoctoral Research Fellow to work with Professor J V Forreste (Department of Ophthalmology) and Dr L A Glover (Department o Molecular and Cell Biology) and collaboratively with Dr S Lightman (Institute of Ophthalmology, University of London), on a project supported by the Fight for Sight Foundation.

The general aim of the project is to develop a molecular model system to study diabetic retinopathy which arises as a consequence of chronic damage to the retinal cells resulting from prolonged hyperglycemia.

The expression of several key genes including oncogenes and growth factor genes will be monitored in a model retinal angiogenic systems by a number of techniques including Northern and Southern blotting and *in situ* hybridisation. The effect of serum factors such as high glucose on the level of specific gene expression will be assessed.

The successful candidate would join a large team of research scientists working on related topics. Previous experience in Molecular Biology and Tissue Culture techniques would be an advantage.

This is a three year post, tenable from 1st July, 1990 or date to be arranged.

Salary on the Range IA Scale £10,458 – £16,665 per annum for Research and Analogous Staff with appropriate placing (salaries under review).

Further particulars are available from the Personnel Department (0224 273500). Applications by letter and CV (two copies) to the **Personnel Department, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX** by 22nd June 1990 quoting reference LG/027.

(3764)A

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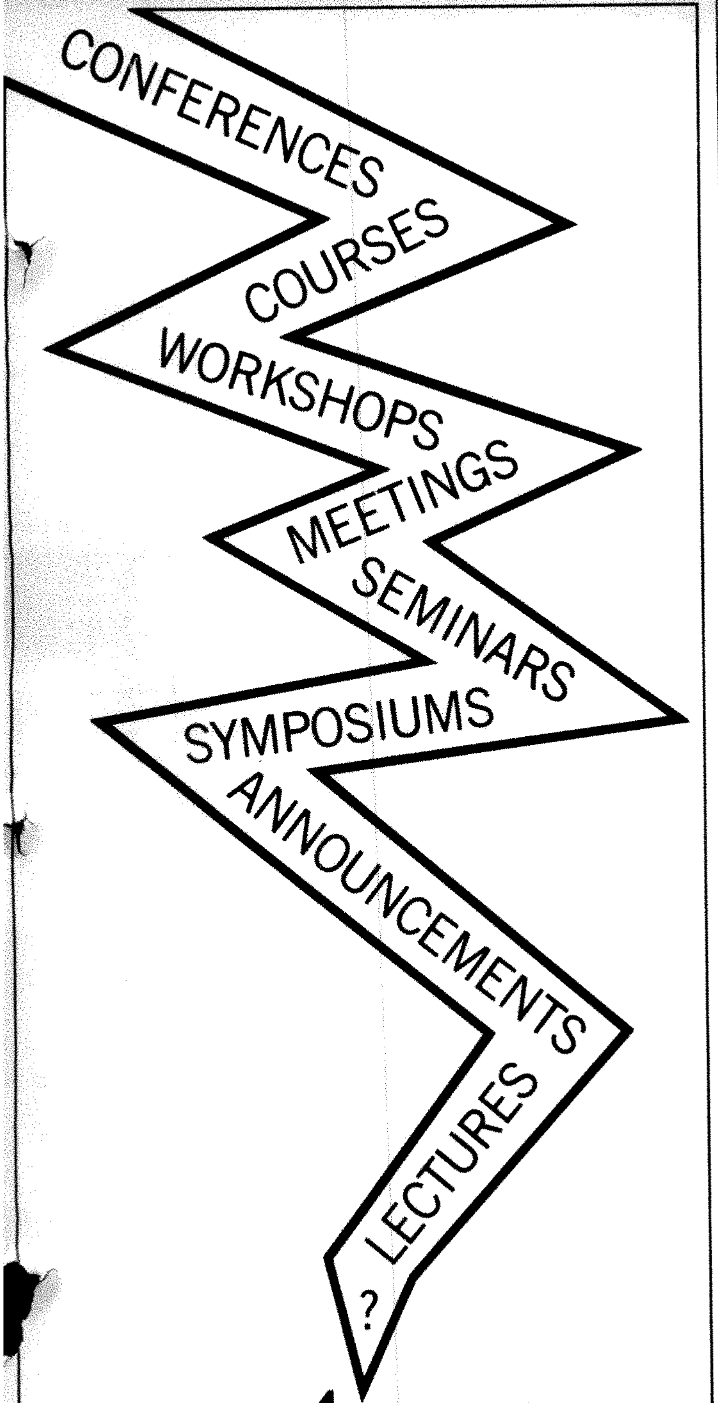
(3634)F



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LECTURESHIP IN ORGANIC CHEMISTRY

UEA NORWICH

Applications are invited for an NAAS Lectureship in the Sector of Organic Chemistry in the School of Chemical Sciences, UEA, tenable from 1st October 1990.

Candidates with interests in **bioorganic chemistry and/or synthetic methodology** will be preferred.

The initial salary will be on the lecturer A scale, £11,399–£16,755, or on the lecturer B scale, £17,455–£22,311.

Applications (three copies), which should contain a full curriculum vitae including exact date of birth, together with the names and addresses of three persons to whom reference may be made, should be lodged not later than **15th June 1990**, with the **Administrative Secretary, University of East Anglia, Norwich, NR4 7TJ**, from whom further details may be obtained, quoting ref. CHE/90/02.

UEA is an equal opportunities employer. (3738)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
INFECTIONS & IMMUNITY GROUP
POSTDOCTORAL SCIENTIST
(Ref: IV/0298)

Applications are invited for a short-term (three years) postdoctoral appointment with Dr Jonathan Stoye in the Division of virology which is studying the mechanisms and consequences of retrovirus integration. Projects include cloning and characterizing the mouse retroviral restriction locus (Fv-1; J.Virol., *63*, 1763) and detailed analysis of the mouse 'hairless' mutation, which is the result of a retrovirus integration (Cell, *54*, 383).

The position is available immediately. Informal enquiries may be made by telephoning Dr Stoye on 081-959 3666 Ext 2140. The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA,** not later than 12th July 1990, quoting reference number IV/0298.

MRC
Medical Research Council

The MRC is an Equal Opportunities Employer (3712)A

POSTDOCTORAL POSITIONS IN YEAST MOLECULAR GENETICS

Two postdoctoral positions are available immediately to study unusual mechanisms of genetic regulation in retroviral-like transposable elements of yeast. The projects involve molecular and biochemical analysis of two unusual phenomena: (1) highly efficient site-specific translational frameshifting analogous to, but mechanistically distinct from, the *gag-pol* frameshift of retroviruses (CELL, in press); and (2) negative regulation by transcription by a novel two-component transcriptional silencer (in preparation). The laboratory takes an approach involving highly directed *in vitro* mutagenesis, analysis of transacting protein factors, and classical yeast genetics to dissect the regulation of expression of Ty elements. Preference will be given to candidates with experience in molecular biology or biochemistry. Funds are available for a minimum of four years.

THE UNIVERSITY OF MARYLAND
COLLEGE PARK CAMPUS
Agricultural Biotechnology Center

Send a curriculum vitae to:
Dr. Philip J. Farabaugh,
Department of Biological Sciences,
University of Maryland,
Baltimore County,
Baltimore, MD 21228.

The University of Maryland is an Equal Opportunity Employer. (NW5358)A

DEPARTMENT OF VISUAL SCIENCE

Institute of Ophthalmology, Judd Street, London WC1H 9QS
(Tel: 071-387 9621)

POSTDOCTORAL RESEARCH ASSISTANTS

1. **ELECTROPHYSIOLOGIST**: This post is for 3 years and is intended to investigate the membrane currents underlying the rhythmic activity of thalamocortical cells using brain slice and patch clamp techniques.
2. **NEURONAL MODELLING**: This post is for 2 years and involves the development of computer simulations of the different firing modes of thalamocortical cells and analysis of their dynamics.

These posts are funded by The Wellcome Trust with salary in the range £14,703-£16,014 + £1,767 LA. Applications with curriculum vitae and names of 2 referees should be sent to **Dr V Crunelli** at the above address, from whom further particulars may be obtained (tel. ext. 233)

3. **NEUROPHYSIOLOGIST**: This post is for 3 years and is funded by the MRC — project entitled "Transmitters of visual and somatosensory afferents to the superior colliculus". Experience of neurophysiological and/or neuro-pharmacological techniques (especially in relation to amino acid transmitters or sensory systems) would be an advantage although not essential.

Salary for this post in the range £12,381-£13,527 + £1,767 LA. Applications, including curriculum vitae, should be sent to **Dr T E Salt** at the above address (tel. ext. 231) (3737)A

THE PETROLEUM SCIENCE AND TECHNOLOGY INSTITUTE
EDINBURGH AND HERIOT-WATT UNIVERSITIES

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a three-year appointment as a geochemist to join a research group directed by Professor Brian Smart, and Drs. Ian Main, Graham Shimmield and Stephen Elphick. The project entitled "The Effects of Combined Changes in Pore Fluid Chemistry and Stress State on Reservoir Permeability", forms part of the core research programme of the recently founded Petroleum Science and Technology Institute. Using state-of-the-art analytical and rock mechanics technology, a series of rigs to simulate *in situ* petroleum reservoir conditions will be constructed.

The successful candidate will be employed on the geochemical aspects of this study in close collaboration with Dr Graham Shimmield. Experience of analytical and inorganic chemistry is essential; knowledge of HPLC, IC and FIA techniques would be advantageous. Specific responsibilities will include the establishment of on-line geochemical analysis of changes in pore fluid chemistry under applied stresses. Rock fabric studies will be carried out with ion probe and SEM techniques for which appropriate training will be given.

The salary will be on the research staff grade 1A (£10,458 to £16,665) depending on age and experience. Applicants should submit their c.v. and the names of two referees to Mrs Patricia Stewart, Secretary to the Petroleum Science and Technology Institute, Grant Institute, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JW, by the 6th June, 1990. PLEASE QUOTE REFERENCE NO. 5773.

(3649)A

CHAIRMAN, DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

University of Florida College of Medicine
Gainesville, Florida

Nominations and applications are requested for the position of Professor and Chairman of the Department of Pharmacology and Therapeutics of the University of Florida College of Medicine. The College seeks an individual who possesses and has demonstrated strong leadership abilities. The candidate should have an established and continuing record of major research productivity. The candidate should have a commitment to the further development and strengthening of the research and teaching programs of the Department. Applications and nominations are invited at this time. Outstanding candidates with M.D. and/or Ph.D. degree will be considered. Applications including curriculum vitae, bibliography, and the names and addresses of at least three references should be sent by September 1, 1990 to:

James E. McGuigan, M.D., Chairman
Search Committee for Pharmacology and Therapeutics
Chairman, Department of Medicine
University of Florida College of Medicine, J-277
Gainesville, FL 32610

An Equal Opportunity/Affirmative Action Employer. (NW5307)A

UNIVERSITY OF ABERDEEN
DEPARTMENTS OF MEDICINE AND THERAPEUTICS AND PATHOLOGY

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to join an established project group studying glomerulonephritis. The project is concerned with possible therapy for experimental glomerulonephritis using novel immunosuppressive drugs. The successful applicant will use novel immunosuppressants to treat the disease, apply cell culture techniques, use flow cytometric analysis and implement several immunological assays.

Applicants should hold or expect to obtain an Honours Degree in Biological Sciences preferably with a background in immunology or biochemistry. The post is tenable for 12 months commencing 1 October 1990 with salary placement on the scale £9,816-£10,458 (pay award pending) depending on qualifications and experience.

Interested scientists are invited to contact Dr Alison MacLeod (0224 681818 ext 53013) or Dr Angus Thomson (ext 52851) on an informal basis.

Further particulars are available from the Personnel Office (0224 273500). Applications by letter and CV (two copies) including names of three referees to the **Personnel Office, University of Aberdeen, Regent Walk, Aberdeen, AB9 1FX** quoting reference LG/026 by 22nd June 1990. (3763)A

UNIVERSITY OF BRISTOL

TEMPORARY LECTURER (2 YEARS) IN PHYSIOLOGY

Following the award of an MRC Senior Research Leave Fellowship to Dr Jonathan Ashmore, the Department of Physiology is seeking applications for a two year appointment from 1st October 1990. The position will cover undergraduate teaching of physiology, primarily of the senses, to science, medical and veterinary students. The position also provides opportunities in an enthusiastic and dynamic environment for research collaboration on a wide range of projects, including those on hearing mechanisms in Dr Ashmore's own laboratory.

Salary: £10458–£14169 on the Lecturer Grade A scale (currently under review).

Further particulars may be obtained from the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH**, to whom applications, including curriculum vitae and names of three academic referees, should be sent by 22nd June 1990. Please quote reference CYF/238. (3727)A

MEDICAL MOLECULAR BIOLOGY UNIT
AND BLOOMSBURY RHEUMATOLOGY UNIT
UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
MOLECULAR IMMUNOLOGY

POST DOCTORAL RESEARCH ASSISTANT and GRADE D TECHNICIAN

Applications are invited for the above positions to work with Dr. David Latchman and Dr. David Isenberg on a project studying the molecular mechanisms producing over-expression of heat shock proteins in human auto-immune diseases such as rheumatoid arthritis as well as the role of this over-expression in producing the observed immune response to these proteins. The project will involve both molecular biology and immunology techniques. Experience in either of these areas would therefore be an advantage but is not essential as training will be provided. The posts are supported by the Arthritis and Rheumatism Council for three years. Salary for the post-doctoral position is on the RA1A scale up to £13,527 plus £1767 London Allowance depending on age and experience. In addition the successful applicant will receive an enhancement premium equivalent to four points on the RA1A salary scale (approximately £2,500 per annum). Salary for the technical position is on the TD Scale up to £10,632 plus £1,750 London Allowance. Informal enquiries may be made to Dr. Latchman (071 380 9343) or Dr. Isenberg (071 380 9230).

Applications with the names of two references to **Dr. D. S. Latchman, Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street**, London **W1P 6DB**. *Equal Opportunities Employer.*

(3722)A

ENDOWED CHAIR
CHAIRMAN, NEW GENETICS DEPARTMENT

St. Jude Children's Research Hospital, a private research institute, is creating a new Department of Genetics. The chairperson will be expected to direct an internationally recognized research program in contemporary genetics and will shape the future of genetics research within the institution through new faculty recruitment and by contributions to its overall scientific leadership. The Department of Genetics will be located in a new 240,000 square foot research building to be completed in early 1991, which will also accommodate ongoing basic science programs in Virology and Molecular Biology, Biochemistry, Immunology, Pharmacology, and Tumor Cell Biology, leaving ample space for further future expansion. The Chairman's salary will be provided through committed endowment funds, together with additional resources commensurate with the needs of a dynamic and ambitious individual. Opportunities exist to interact with strong clinical programs in pediatric Hematology/Oncology and Infectious Disease. Applicants should submit a curriculum vitae and the names and addresses of four references to:

James N. Ihle, Ph.D.
Chairman, Department of Biochemistry
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, Tennessee 38105

St. Jude Children's Research Hospital is an Equal Opportunity Employer (NW5325)A

Postdoctoral Position

available in the Department of Neurology, University of California San Francisco to study signal transduction mechanisms involved in neural differentiation. Grant support for this project has been obtained for at least 3 years. Interested applicants should send curriculum vitae and 3 letters of reference to: **Robert Messing, M.D., Building 1, Room 101, San Francisco General Hospital, San Francisco, CA 94110.** *EOE/AA.* (NW5368)A

POSTDOCTORAL RESEARCH ASSOCIATE

Postdoctoral position: molecular biology and genetic transformation in marine algae. Ph.D. and experience in molecular biology techniques and cell culture required. Experience with culture of marine algae desirable. Send résumé and three letters of recommendation by 20 June 1990 (*non-citizens must include current visa status*) to: **Dr. Subhash C. Minocha, Department of Plant Biology, University of New Hampshire (UNH), Durham, NH 03824. Telephone: 603-862-3840.** *UNH is an Affirmative Action/Equal Opportunity Employer.* (NW5374)A

WESSEX REGIONAL GENETICS LABORATORY
GRADE B CYTOGENETICIST
(PRINCIPAL)

A vacancy has arisen for a Grade B Cytogeneticist to take charge of one of the cytogenetic sections of a large diagnostic laboratory. While it is the policy of the laboratory to rotate principal cytogeneticists among sections, the immediate need is for an individual to take charge of the leukaemic section and priority will be given to applicants with special expertise and interest in the cytogenetics of malignancies.

Applicants must have the Diploma of The Royal College of Pathologists in Cytogenetics or an equivalent qualification.

Salary on the scale of £17,996 – £23,682. Further information may be obtained from Dr Patricia Jacobs or Dr Nina Gregson. Telephone Salisbury (0722) 336212 Ext. 669.

For an Application Form and Job Description please WRITE to the

Personnel Department,
Odstock Hospital,
Salisbury, Wiltshire
SP2 8BJ.

Closing date: 15th June 1990.

(3658)A

Research Scientist
Edinburgh

A 6 year tenure track position is available for a young scientist to set up their own research programme within the ICRF Molecular Pharmacology Group in Edinburgh.

The overall aims of the group are to understand the molecular and genetic factors which determine the sensitivity of normal and tumour cells to toxic and mutagenic chemicals. At the present time the group has particular interest in using molecular and genetic approaches to identify important genes in these processes and also in the study of transcriptional regulation of gene expression. The work of the Research Scientist however need not be on these themes and could be on any aspect of prokaryotic or eukaryotic molecular biology or molecular genetics.

For further information please contact Dr. C. R. Wolf, Tel: 031-668 3343 or 031-667 1011 ext 2417. Applications consisting of a full C.V. and the names of three referees should be sent to Dr. C. R. Wolf, ICRF Molecular Pharmacology Unit, Department of Biochemistry, University of Edinburgh, Hugh Robson Building, George Sq, Edinburgh EH8 9XD.

Smoking is actively discouraged.

Imperial Cancer
Research
Fund

(3736)A

University of Bradford

DEPARTMENT OF ENVIRONMENTAL SCIENCE

RESEARCH ASSISTANT

IN ENVIRONMENTAL GEOCHEMISTRY

INORGANIC TRACE POLLUTANTS AND MICRONUTRIENTS IN SOILS OF TROPICAL ECOSYSTEMS WITH SPECIAL REFERENCE TO VENEZUELA

Applications are invited for the post of Research Assistant in the Department of Environmental Science and which represents an unusual opportunity to work in a new research field of great potential importance to tropical areas. The post is available for a period starting as soon as practicable and ending 30 April 1993 to work under the direction of Professor B E Davies. Applicants should possess a good Honours degree in an area such as soil science, geochemistry, geology or analytical chemistry. Persons trained in ecology may also be suitable but an adequate chemical background is needed. The work will involve field sampling in Venezuela. A knowledge of the Spanish language, while not essential, would be advantageous. Salary on scale £9816 – £12381 pa (award pending). Superannuable. Application forms and further details from the **Senior Assistant Registrar Personnel, Ref RA/ES/BD/N, University of Bradford BD7 1DP**. Tel 0274 733466 ext 224. *An equal opportunity employer.* Closing date: 22.6.90. (3765)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY

Post-doctoral Research Worker

Applications are invited for a molecular biologist to work on an MRC project entitled "Expression of recombinant antigens in salmonellae as combined vaccines".

The successful candidate will work on optimising of expression of recombinant antigens in live attenuated salmonellae so as to elicit maximal immune responses. The post is funded until November 1992 in the first instance and the salary is on the University Salary Scale (£10458-£15372) depending on age and experience.

Applications with c.v. and names and addresses of two referees should be sent to **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP.** Further details may be obtained from Dr. C. E. Hormaeche (Tel: 0223 333330). Please quote Ref. 11498. (3746)A

HARVARD UNIVERSITY

ASSISTANT PROFESSOR

CELLULAR AND DEVELOPMENTAL BIOLOGY (CDB)

The Department of Cellular and Developmental Biology intends to make an appointment at the assistant professor level effective September 1992. Our department covers a broad range of topics in cellular biology, developmental biology, neurobiology and molecular genetics. We are seeking outstanding candidates in any of these fields. We are particulary interested in making an appointment in the areas of:

★ **Plant molecular biology** ★ **Developmental genetics** ★ **Protein sorting**

However, we will consider exceptional applicants in other fields as well. We strongly encourage applications from women and minority groups. Applications should include curriculum vitae, reprints of publications, and a statement of present and future research plans. Complete applications and three letters of recommendation, *solicited by the applicant*, should be received no later than *October 1, 1990.* **CDB Search Committee, Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138**, (617) 495-2327.

Harvard University is an Affirmative Action/Equal Opportunity Employer.

(NW5363)A

UNIVERSITY OF FRIBOURG SWITZERLAND
INSTITUTE OF PHYSIOLOGY

Applications are invited for a **postdoctoral position** for 2 years. Candidates with strong interest and experience in the fields of behavioural techniques, microelectrophysiology, computer-analysis and -control are particularly welcome. The ongoing research project is centered on motor control, including the analysis of movement parameters, EMG and single unit activity in monkeys. Salary (Swiss National Science Foundation) is approximately SFr. 40'000.- p.a.

Send applications with CV and if possible names of referees to **Professor M. Wiesendanger, Institut de Physiologie, rue du Musée 5, CH-1700 Fribourg, Switzerland.** (W7393)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
DEPARTMENTS OF BACTERIOLOGY AND MEDICINE
INFECTIOUS DISEASES UNIT

RESEARCH OFFICER

Applications are invited for this 2 year post to work with an active research group investigating septic shock. We are concerned both with elucidating the mechanisms and developing novel therapeutic strategies, with a particular emphasis on the role of cytokines; the proramme already has an active clinical component.

The post is available immediately. Applicants should have experience in medical and/or veterinary research, ideally in immunology or cell biology.

Salary will be in the range £9816-£12381 plus £1767 London Allowance pa.

Application forms and further particulars are available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 ONN (tel: 081-740 3204)** quoting ref: AMPE.

Closing Date: 14 June, 1990. (3714)A

THE UNIVERSITY OF BIRMINGHAM
RENAL RESEARCH LABORATORY

RESEARCH FELLOW

Funded by National Kidney Research Fund (post-doctoral Research Fellow) to join a group studying glomerular endothelial injury in the Haemolytic Uraemic Syndrome and Vasculitis. Research is aimed at investigating mechanisms of injury in cultured glomerular endothelial cells. Experience in immunochemical and/or molecular biological techniques desirable.

The post is initially for two years, wtih possibility of extension. Salary on a scale £10,458-£16,665 (currently under review).

Application forms (returnable by 4 July 1990) and further particulars available from **The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT.** Telephone 021 414 6483 (24 hours). Quote ref M12062.

The University is an equal opportunities employer. (3651)A

ASSISTANT PROFESSOR

Department of Chemistry
The American University

A one year temporary, non-tenure track position is available starting in August, 1990. The position may be renewable for a maximum of three years of service. Applicants should be qualified to teach in the areas of Biochemistry and Pharmacology. The successful candidate will teach both on campus and in an off-campus master's program, as well as provide graduate advising and supervision of master's thesis research. Qualifications: Ph.D. required; post-doctoral experience desirable. Position subject to final budgetary approval.

Applicants should submit a curriculum vitae, an outline of research interests, a list of publications and three letters of reference to **Dr. James E. Girard, Chair, Department of Chemistry, The American University, 4400 Massachusetts Avenue, N.W., Washington, D.C. 20016.** Application deadline: June 15, 1990.

The American University is an EEO/AA university. Minority and women candidates are encouraged to apply. (NW5377)A

UNIVERSITY OF LEICESTER

Department of Genetics

RESEARCH ASSISTANT

Applications are invited for this post supported by the Medical Research Council to work on yeast protein kinases. The successful candidate will join a large group studying signal transduction pathways in yeast by genetical and biochemical methods. The post is for three years and the salary is in Range RA IB (£11,399–13,495) p.a.

Applications including a C.V. and the names and addresses of two referees should be sent to: **Dr. E. Orr, Department of Genetics, University of Leicester, University Road, LEICESTER, LE1 7RH** by 30 **June 1990.** Telephone (0533) 523424. (3755)A

SCHERING

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Please forward your c.v., a list of publications, and a summary of your former research activities marked "confidential" to:

Schering Aktiengesellschaft
Personalabteilung FO
Müllerstr. 170-178, D-1000 Berlin 65

(W7379)A

POSTDOCTORAL POSITIONS
RESEARCH INSTITUTE OF SCRIPPS CLINIC
LA JOLLA, CALIFORNIA

Competitive research positions are available for studies and training with a group of scientists interacting in programs covering:

Central Nervous System-Immune System Interaction
and
Central Nervous System Effects of Retrovirus Infections

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Michael B.A. Oldstone: Viral pathogenesis, molecular mimicry, transgenic mice
Floyd Bloom: Molecular neurobiology
J. Lindsay Whitton: Molecular basis of CTL induction and recognition
Michael Buchmeier: Viral CNS pathogenesis and demyelination
George Koob: Neuropharmacology and behaviour
Greg Sutcliffe: Molecular neurobiology
Rob Milner: Super gene families and astrocyte differentiation
Jim Hogle: Structure-function study of viruses and viral receptors
Ian Wilson: Structure-function study of antibody-antigen complexes, and cytokine and viral receptors

Letters of application including curriculum vitae, a statement of research interests and future goal(s), names and addresses of three referees should be sent to:

Michael B.A. Oldstone, M.D.
Research Institute of Scripps Clinic
10666 N. Torrey Pines Road
La Jolla, CA 92037

The NIMH training award is restricted to candidates having U.S. citizenship or permanent resident status. (NW5367)A

Toxicologist/Chemist/ Product Regulations

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Hewlett-Packard, located in the Pacific Northwest, offers an excellent employment and living environment reputed throughout the industry. Please send your resume to: **Janet Flaherty, Personnel, Hewlett-Packard Company, 1000 N.E. Circle Blvd., Corvallis, OR 97330.** Hewlett-Packard Company is an Equal Opportunity/Affirmative Action Employer.

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(NW5339)A

ROYAL HOLLOWAY AND BEDFORD
NEW COLLEGE
University of London

MONOCLONAL ANTIBODY UNIT
Scientific Officer

A well-qualified Scientific Officer with considerable experience in monoclonal antibody production and immunological techniques is required to run the monoclonal antibody unit. The Unit is a Life Sciences research facility housed within the Biochemistry Department. Its major function is the production and growth of monoclonal antibodies for the various research groups within the School of Life Sciences. The successful applicant will thus interact with a number of major research projects mainly in the area of cell recognition and neurobiology and plant biochemistry and molecular biology. The appointment will be on the Academic related research scales IA or IB (£11,583-£15,294 currently under review) depending on experience and qualifications.

Further details and application forms are available from the **Personnel Office, RHBNC, Egham Hill, Egham, Surrey TW20 0EX.** Tel: 0784 443027. Informal enquiries to Dr. P. W. Beasley (0784 443546). Applications (two copies) should be submitted by June 30th 1990. (3762)A

UNIVERSITY COLLEGE DUBLIN
DEPARTMENT OF INDUSTRIAL MICROBIOLOGY

Applications are invited for a full-time permanent position of Assistant Lecturer/College Lecturer in the above Department. Applicants should have a special interest in one of the following areas — microbial biotechnology, microbial physiology, pharmaceutical microbiology, environmental microbiology or a related area. In addition to teaching, intending candidates should have a strong commitment to research including collaborative work.

Salary Scale

Assistant Lecturer: Ir£11,595 - Ir£18,674.
College Lecturer: Ir£18,046 - Ir£25,420.

Entry point on the relevant scale will be in accordance with qualifications and experience. There is a non-contributory pension scheme.

Application Procedure

Prior to application, further information (including application procedures), should be obtained from the **Secretary, University College Dublin, Belfield, Dublin 4**. Telephone enquiries: **(0001) 693244, ext. 1413.**

The closing date for receipt of completed applications for the above post is Friday 6th July 1990. (3771)A

UNIVERSITY OF THE WEST INDIES
Cave Hill Campus, Barbados

Applications are invited for the following post:

SENIOR LECTURER/ LECTURER IN ECOLOGY
Department of Biology

Preference may be given to candidates who work at the interface between ecology and evolutionary genetics, or who have an active research interest in marine ecology or conservation ecology. The successful applicant is expected to assume duties as soon as possible.

Salary Scales: Lecturer BDS$41,316 × 1,776 — 51,972 (Bar) × 1,776 — 62,628 pa. Senior Lecturer BDS$54,840 × 1,776 — 65,496 × 1,920 — 71,256 (Bar) × 1920 — 75,096 p.a. Up to five full economy class passages plus baggage allowance of US$1,200 on appointment and normal termination. Special allowance up to US$400 for shipment of academic books and teaching/research equipment on appointment. Unfurnished accommodation at 10% of basic salary, or optional housing allowance of 20% of basic salary to staff making own housing arrangements. UWI contribution of equivalent of 10% of basic salary to Superannuation Scheme. Annual Study and Travel Grant for self, spouse and up to three children. Book Grant up to BDS$850 per annum. Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status and the names and addresses of three referees should be sent as soon as possible to the Campus Registrar, University of the West Indies, PO Box 64, Bridgetown, Barbados. The University will send further particulars for this post to all applicants. These particulars may also be obtained from Appointments (37919), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF UK. (W7200)A

Medizinische Universität zu Lüback
Abteilung Klinische Rheumatologie

RESEARCH ASSOCIATE

Applications are invited for the post of a Research Associate. The initial appointment will be for 2–3 years at BAT IIa/BAT Ib, depending on research experience. The candidate will join a project focusing the mechanisms of cytokine activation and their effects on the human immune system, especially in autoimmune diseases. The position is part of a project granted by the Bundesministrium für Forschung und Technologie. The candidate should have special experience in molecular biology and protein chemistry. Applications including a curriculum vitae, list of publications and the names of 2 referees, should be sent to:

Prof. Dr. W.L. Gross, Direktor der Abteilung Klinische Rheumatologie, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, 2400 Lübeck 1, Fed. Rep. Germany, or Dr. J. Kekow, Scripps Clinic, Department of Molecular and Experimental Medicine, 10666 North Torrey Pines Road, La Jolla, Ca. 92037, U.S.A.

Deadline is July 31, 1990

(NW5381)A

ST MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology & Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR GENETICS

Two Research Assistant posts are available in our Department to work on the following projects:

1. TRANSGENIC MOUSE MODEL FOR DOWN'S SYNDROME. The successful applicant would join a group using various chromosome transfer methods to transfect EC cells and extend the work with ES cells. (This project is in collaboration with Dr R Lovell-Badge, NIMR, London).
2. CHARACTERISATION OF A TRANSGENIC INSERTIONAL MUTATION. We have cloned the disrupted gene from a dysmorphic mouse and the successful applicant would complete sequencing and expression analysis of this clone.

We are an equal opportunity employer. For further details telephone Dr Peter Scambler (071-723 1252 ext. 5499). Applications in the form of a full CV, with names and addresses of 2 referees should be sent to **Personnel Department** at the above address. (3718)A

TENURE TRACK APPOINTMENT IN ENVIRONMENTAL BIOLOGY

The Environmental Science and Engineering Interdepartmental Program and Department of Environmental Health Sciences, UCLA School of Public Health. Tenure track faculty member in the general area of environmental biology. Specific research areas could be in ecology, ecotoxicology, conservation biology, impacts of pollutants on plant or animal systems, or related areas. Awareness of and interest in policy related implications of environmental biology will be important, especially in relation to teaching and student guidance. Candidates should have an earned doctorate and several years experience. The level of this appointment is open; rank and salary will be commensurate with qualifications. The selected individual will serve as one of four core faculty members in the Environmental Science and Engineering Program, which is entirely at the doctoral level. UCLA is an affirmative action/equal opportunity employer.

Interested applicants should submit resumes by September 1, 1990 to **Chair, Search Committee for Environmental Biologist, Environmental Science and Engineering Program, School of Public Health, University of California, Los Angeles, CA 90024.** (NW5353)A

MOLECULAR BIOLOGY POSITIONS

Applications are invited for two tenure-track positions at the rank of assistant professor in Microbiology in the Fulbright College of Arts and Sciences at the University of Arkansas, Fayetteville. (Continuation of search announced in the *ASM News*, March, 1990.) Candidates must have strong postdoctoral research experience, expertise in molecular biology, and ability to teach and direct the work of B.S., M.S., and Ph.D. microbiology majors. Salary and start-up support are nationally competitive. Submit letter of application, curriculum vitae, statement of teaching and research goals, and arrange to have three letters sent to: **Dwight E. Talburt, Department of Botany and Microbiology, the University of Arkansas, Fayetteville, AR 72701.** *The University of Arkansas is*

GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva molecular biology research institute of one of the world's most successful healthcare groups is recruiting a

CELLULAR IMMUNOLOGIST

to investigate the role of adhesion molecules in inflammation

The successful applicant will have a leading role in an expanding research group of molecular biologists, biochemists and cell biologists working in this field. The ideal candidate will have several years of relevant experience and a proven track record of research in cell biology or cellular immunology.

The Glaxo Institute for Molecular Biology carries out long term research to support the company-wide effort to discover effective new medecines. It provides a dynamic research environment, and encourages the publication of scientific results as well as collaborative projects with academic groups.

We offer an excellent salary and benefits package and opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your Curriculum vitae with a list of publications and the names of three referees to: Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W7381)A

MARINE BIOLOGIST

with background in marine birds and/or mammals

MOSS LANDING MARINE LABORATORIES (MLML)

is seeking a tenure track, probationary appointment at the **Assistant** or **Associate Professor** level to provide quality undergraduate and graduate instruction, and to pursue a vigorous research program on some aspect of sea birds and/or marine mammals. Teaching responsibilities include courses in marine birds and mammals and statistics. A Ph.D. is required. The salary range for Assistant Professor level is $30,276-$41,844; Associate Professor level is $38,112-$52,896/academic year. The position may be filled as early as Spring 1991.

Moss Landing Marine Laboratories is located on Monterey Bay, an area noted for abundant, diverse populations of sea birds and marine mammals. The Laboratories are operated by a consortium of California State University campuses including Fresno, Hayward, Sacramento, San Francisco, San Jose, and Stanislaus.

Send CV, representative reprints and the names of three referees by September 1, 1990 to **Dr. John H. Martin, Director, MLML, P.O. Box 450, Moss Landing, CA 95039.**

The University is especially interested in hiring faculty members who are aware and sensitive to the educational goals and requirements of an ethnically and culturally diverse population. An Equal Opportunity/Affirmative Action/Title IX employer.

(NW5344)A

FACULTY POSITIONS IN THE MOLECULAR PHARMACOLOGY OF CANCER

The Department of Pharmacology and the Lineberger Cancer Research Center of the University of North Carolina at Chapel Hill are seeking to recruit one or more outstanding individuals to tenure track positions in the area of basic research related to cancer. Positions are available immediately, and rank will be commensurate with ability and experience.

Preference will be given to individuals with demonstrated competence in applying modern techniques of molecular biology, biochemistry or biophysics to fundamental problems in cancer pharmacology, and molecular oncology. Potential areas of interest would include, but not be limited to, the following, regulation of gene expression by oncogenes or growth factors; signaling mechanisms involved in growth control; molecular bases of drug resistance; mechanisms of action of novel anticancer drugs.

Successful candidates will be expected to establish well-funded independent research programs, to participate in the graduate and professional educational activities of the Department, and to interact closely with members of the Lineberger Cancer Research Center.

Applicants should send a curriculum vitae, a statement of research plans and the names of three referees to:

R. L. Juliano, Ph.D.
Professor and Chairman
Department of Pharmacology
CB # 7365, 1106 Fac. Lab Office Bldg.
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7365

Women and minority candidates are encouraged to identify themselves. Candidates are urged to apply prior to July 15, 1990. The University of North Carolina at Chapel Hill is an equal opportunity/affirmative action employer.

(NW5359)A

UNIVERSITY OF TORONTO
ASSISTANT PROFESSOR — PATHOLOGY

The Department of Pathology invites applications from innovative and energetic candidates for the position of Assistant Professor (Research) from September 1st, 1990. Initial appointment will be for three years, renewable for a further two years.

Applicants should have Ph.D. and/or M.D. degrees, at least two years postdoctoral experience and they must show evidence of strong independent, productive research sufficient to compete for personnel and grant support.

The successful candidate will participate in teaching Experimental Pathology to graduate and undergraduate students, supervise graduate students, develop a vigorous and independent research program and interact positively with current faculty. Preference will be given to applicants with expertise in recombinant DNA technology. Research in the Department includes: molecular mechanisms of carcinogenesis, regulation of complement gene expression and mechanisms in cutaneous phototoxicity.

Applications should include curriculum vitae, list of publications, reprints of publications in last three years and a short statement of research plans. Complete application and three confidential letters of recommendation (solicited by applicant) should be received no later than August 15, 1990 by:

Dr. J.O. Minta
Chair, Search Committee
Division of Experimental Pathology, Room 6308
University of Toronto, Toronto, Ontario
M5G 1L5, Canada

In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. (NW5385)A

The U.S. Department of Commerce (DOC), National Oceanic and Atmospheric Administration (NOAA) invites applications for five positions on the **Program Development and Coordination Staff in the Office of Oceanic and Atmospheric Research (OAR).** The office, located in the Washington D.C. area, provides scientific, programmatic, and policy staff work to the Assistant Administrator for OAR. We are seeking a senior **Physical Scientist** (salary range: $50,300 to 76,000) to serve as staff expert for marine science efforts. Another vacancy exists for a **Physical Scientist** (salary range: $35,800 to 46,500) to serve as a marine science policy analyst. We are also seeking a **Meteorologist** (salary range: $24,700 to 46,500). Persons with expertise in climate, air quality, weather, modelling, instrumentation, or monitoring are invited to apply. Two positions are available for **Oceanographers** (salary range: $24,700 to 46,500). Biological, chemical, geological and physical oceanographers are invited to apply. For further information and specific vacancy announcement numbers, please contact: **Mr. Paul Jordan, NOAA, NCASC Personnel, DC23, 11420 Rockville Pike, Room 40, Rockville, Maryland 20852,** Phone: (301) 443-3434.

DOC is an Equal Opportunity Employer

(NW5386)A

CORTECH, INC.
RESEARCH ASSISTANT MOLECULAR BIOLOGY

Cortech is a Denver-based pharmaceutical research and development company specializing in the areas of immunology, protease biochemistry and receptor pharmacology. We are seeking a research assistant in the area of molecular biology. The successful candidate will have a BS or MS and several years' experience in the field as well as be familiar with several of the following techniques: cDNA library construction and screening, DNA sequencing, expression of foreign proteins in bacteria, protein purification, protein characterization (HPLC, gel electrophoresis, immunochemical, etc.) and peptide mapping. Cortech offers a stimulating environment where a multidisciplinary approach is employed in order to develop new technologies for the treatment of human disease. Cortech is an equal opportunity employer and offers an excellent compensation and benefits package including stock options.

Please direct inquiries to: **Barbara Klipfel, Laboratory Manager, Cortech, Inc., 6840 N. Broadway, Suite F, Denver, CO 80221.** EOE/AA. (NW5366)A

Chair
Department of Pharmacology

The University of Texas Medical School at Houston invites applications and nominations for the position of Chair of the Department of Pharmacology. Responsibilities include directing, managing, planning and continuing development of a department committed to excellence in research and the teaching of graduate students and medical students. The candidate must have a Ph.D., M.D. or equivalent degree from an accredited institution, be qualified for an academic appointment at the rank of Professor, and have demonstrated abilities in administration, teaching and research.

Applications should include a curriculum vitae, a brief statement of current research interests and names of three references. Nominations and applications should be sent by September 15th to: **John H. Byrne, Chair, Pharmacology Search Committee, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77225.**

The University of Texas is an Equal Opportunity Employer
Minorities and women are encouraged to apply.
(NW5395)A

Professor and Director of the Division of Biological Sciences
Harvard School of Public Health

Candidates, who have expertise in the biological sciences as applied to public health problems, are invited to apply for a tenured professorship at Harvard University. Outstanding accomplishment is required in one or more of the following areas: cancer biology, environmental science and physiology, nutrition, toxicology, tropical public health, or related areas. In addition to conducting a strong laboratory research program, the Director should devote approximately thirty per cent effort to heading the Division of Biological Sciences (DBS) at the School of Public Health. The DBS coordinates the recruitment and training of graduate students in the laboratory sciences at the School. The Director should both strengthen one of the programs or departments of the Division, and represent the interests of all the School's laboratory activities. Send curriculum vitae, a statement of research interests, and 3-5 selected reprints to: **DBS Search Committee, Harvard School of Public Health, 677 Huntington Avenue, Building 3-1005, Boston, MA 02115** Women and minority candidates are particularly invited to apply. (NW5371)A

FU | BERLIN

Assistant Research Physiologist

position open in the Neurophysiology Section (Prof. O.-J. Grüsser) of the Department of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33 (West), Germany. Research projects in experimental neurophysiology of the visual, vestibular and oculomotor system. **Requirements:** Ph.D. in neurophysiology, experience with microelectrode recording techniques, computer application and modern neuroanatomical tracer techniques. Submit application (curriculum vitae, list of publications and letter of recommendation) by June 15th, 1990 to **Prof. O.-J. Grüsser, Department of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33.** (W7374)A

POSTDOCTORAL POSITION IN NEUROENDOCRINOLOGY

An outstanding opportunity is available immediately for a Ph.D./M.D. endocrinologist/neuroendocrinologist to undertake research in steroid-brain interactions and neuropeptide regulation related to reproduction. Experience in molecular biology/neurochemistry desirable. Send Curriculum Vitae and names of 3 references to **Dr. Satya P. Kalra, Department of Obstetrics and Gynecology, University of Florida, J-294 JHMHC, Gainesville, FL 32610 USA.** *An Equal Opportunity/Affirmative Action Employer.*

(NW5389)A

PROGRAMME MANAGERS/CONSULTANTS

Protein Engineering. Plant and Microbial Metabolism. Eukaryotic Genetic Engineering.

The Biotechnology Directorate of the SERC is seeking to appoint up to three programme managers for LINK programmes jointly sponsored by SERC, DTI and other Research Councils. The appointees may also be required to act as technical consultants to the Directorate in their specialised fields.

The appointees will play a key role in establishing and managing projects and programmes within the LINK schemes. Coming from a relevant industrial or academic background, the appointees will have strong managerial and organisational skills, and be able to communicate clearly with academics and industrialists at all levels. These posts are challenging, requiring initiative, vision, enthusiasm and commitment.

The areas for which programme managers/consultants are required are:

***PROTEIN ENGINEERING:** A multidisciplinary programme focussed on understanding of relationships between structure and function of proteins, tertiary structure determination and genetic expression of proteins. (REF: BTD/PE)

***EUKARYOTIC GENETIC ENGINEERING:** A programme aimed at increased understanding and control of molecular genetics including development of genetic engineering techniques, novel vector developments, gene expression and other related areas. (REF: BTD/EGE)

***CONTROL OF PLANT AND MICROBIAL METABOLISM:** A programme aimed at improving basic understanding of plant metabolism and metabolism and physiology of micro-organisms, which will facilitate improvements in application of genetic engineering techniques. (REF: BTD/PMM)

The appointments will be on a part-time contractual basis. Period of appointment, duration of contract, location, and daily fees are subject to negotiation. Applications are welcomed from suitably qualified individuals. Academics or industrialists may be appointed on secondment.

Applicants should send a full CV together with the names and addresses of three referees and state the reference number of the post. Applications should be sent to:

Mr Nigel Birch, Biotechnology Directorate, Polaris House, North Star Avenue, Swindon, Wilts. SN2 1ET
to arrive no later than 29 June 1990.

(NW5369)E

The Science and Engineering Research Council

ASSISTANT PROFESSOR

UNIVERSITY OF WESTERN ONTARIO

One Limited Term position (5 years) is available at the Assistant Professor level starting as early as September 1st, 1990. Applicants should possess an M.D. or a Ph.D. in pharmacology, toxicology or a closely related discipline and must have three years of post-doctoral experience. We are particularly interested in highly-qualified individuals who are using techniques of contemporary cell or molecular biology to study pharmacological and/or toxicological problems of long term significance. The candidate is expected to develop an externally funded research program and to participate in undergraduate and graduate teaching. Salary for the position is commensurate with qualifications and experience. The position is subject to budget approval. The deadline for applications is July 15th, 1990.

Applicants should forward a Curriculum Vitae, three letters of reference and a description of proposed research to: **Dr. J. R. Bend, Professor and Chairman, Department of Pharmacology and Toxicology, The University of Western Ontario, London, Canada N6A 5C1**.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and Permanent Residents of Canada. *The University of Western Ontario is an equal Opportunity Employer.* (NW5362)A

UNIVERSITY OF NEWCASTLE UPON TYNE

RESEARCH ASSOCIATE IN AUTOIMMUNITY

Applications are invited for this 2 year post funded by the Wellcome Trust to study the molecular basis of the autoimmune disease, primary biliary cirrhosis. The successful applicant will join a team of basic and clinical scientists who have recently identified several major autoantigens in this chronic liver disease. The group occupies new, well equipped laboratories within the Medical School. Some experience of protein chemistry and/or molecular biology would be an advantage.

Salary will be on the Research Associate Grade 1A salary scale £10,458 – £16,665 or Grade 1B salary scale £9,816 – £12,381 per annum (including an enhancement premium of two increments) depending on qualifications, age and experience.

Potential applicants are encouraged to contact **Dr. S.J. Yeaman** (Tel. 091 222 7433), **Department of Biochemistry & Genetics, The Medical School, Newcastle upon Tyne, NE2 4HH**, to whom applications (including CV and the names and addresses of three referees) should be sent, as soon as possible. (3743)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF MEDICINE

Research Assistant

Applications are invited for a Research Assistant to work on the effects of pressure and flow on the contractile responses of human resistance arteries. The project is for three years, and would be suitable for someone wishing to read for a higher degree. It is funded by the Medical Research Council and the salary is on RA IB scale (£11,399-13,495 p.a.) according to age and experience. The successful applicant will join a research team devoted to small artery research in cardiovascular disease, especially high blood pressure.

Applications, with names and addresses of two referees, should be sent to: **Dr. A. M. Heagerty, Department of Medicine, University of Leicester, University Road, Leicester LE1 7RH, tel: 0533-523183**, from whom further details may be obtained. (3717)A

POSTDOCTORAL ASSOCIATE

FOX CHASE CANCER CENTER

Research involves the study of genetic alterations that occur in human cancer and their underlying mechanisms. Research will utilize a molecular approach to: 1) analyze spontaneously occurring mutations in the EGF receptor; 2) determine role of signal transduction proteins in tumorigenesis. Familiarity with the methods of molecular biology preferred. Submit resume and names of three references to **Dr. Albert Wong, c/o PERSONNEL REPRESENTATIVE — Daw; Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.** (NW5355)A

Postdoctoral Positions in Molecular Virology

To study molecular biology of RNA-dependent RNA replication, gene expression/regulation, RNA-RNA recombination, and host specificity in (+) strand RNA viruses. Send curriculum vitae brief statement of research experience and further goals, and have three reference letters sent to: **Dr. Paul Ahlquist, Institute for Molecular Virology, University of Wisconsin, Madison, WI 53706**. Telephone: 608-262-4540. *Equal Opportunity/Affirmative Action Employer.* (NW5376)A

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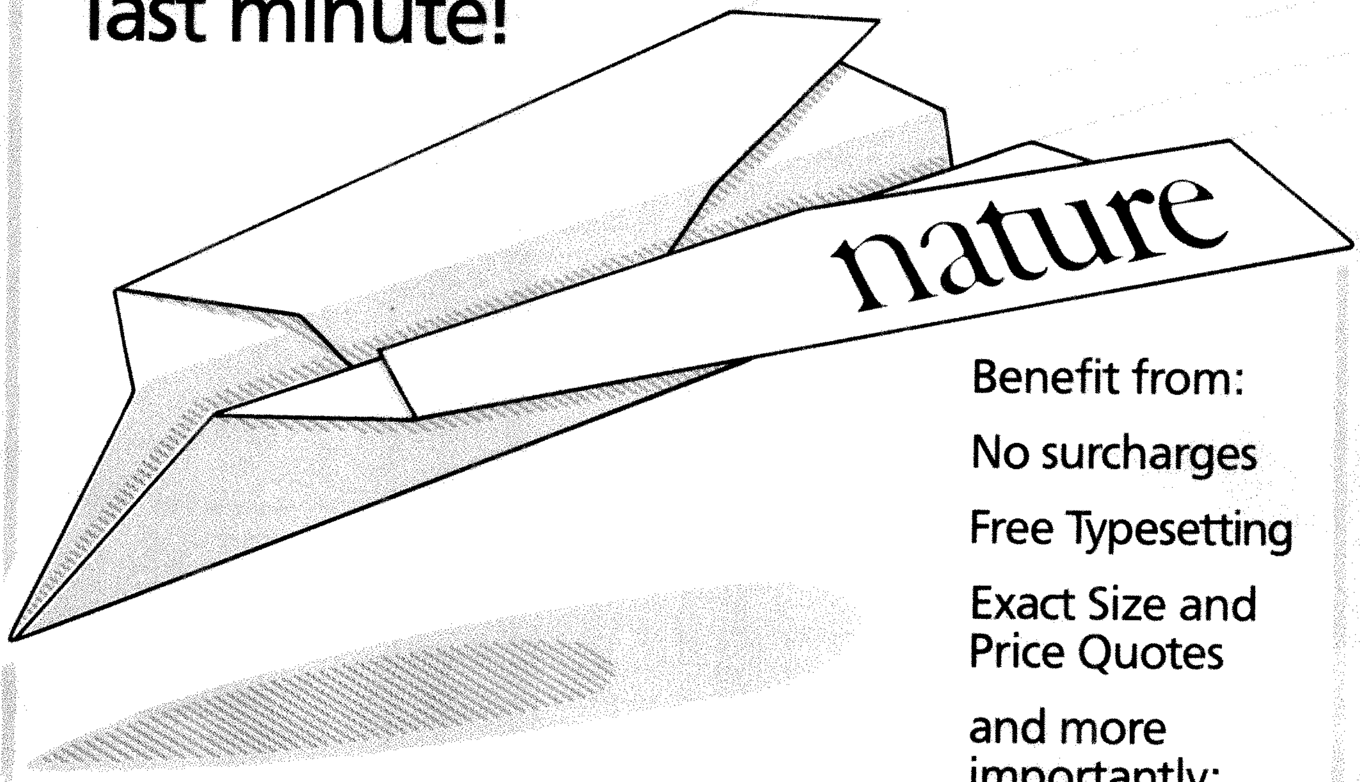

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SCHERING

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We are a leading chemical-pharmaceutical company in West-Germany with 25,000 employees worldwide. At our headquarters in Berlin we employ 7,000 people. Our pharmaceutical branch is committed to the development of new therapeutic agents through basic research.

For an interdisciplinary project on "Sexual Hormones and Hemostasis" we are looking for a young scientist for a two-year period (extension for a further year is possible) as a

Post-Doc: M. D. or PH. D.

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Please forward your c.v., a list of publications, and a summary of your former research activities marked "confidential" to:

Schering Aktiengesellschaft
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Müllerstr. 170-178, D-1000 Berlin 65

(W7377)A

PROGRAM IN EXCELLENCE IN MOLECULAR BIOLOGY OF THE CARDIOVASCULAR SYSTEM

NEW INVESTIGATOR POSITIONS

Molecular Biology of Ion Channels

The NHLBI of the NIH has awarded the University of Cincinnati Medical Center a new program providing unique opportunities for training and research in the Molecular Biology of the Heart and Lung.

Postdoctoral assistant or junior faculty positions are now available in the Component on the Structure and Function of Ion Channel Proteins.

Candidates with molecular genetic skills possessing a Ph.D. and/or M.D. degree, who wish to address problems associated with ion channels of heart, smooth muscle, and brain, are encouraged to apply.

Beginning stipends for recent graduates are at a minimum of $25,000 including benefits, and progresses to $37,000 or higher in final years.

Applicants should submit a C.V. and the names of three references to **Dr. Arnold Schwartz, Department of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267-0575.** If you wish further information, telephone 513-558-2400. FAX number 513-558-1778.

Equal Opportunity Employer

(NW5373)A

ASSISTANT PROFESSOR/ASSOCIATE DIRECTOR
INSTITUTE FOR ROCK MAGNETISM
UNIVERSITY OF MINNESOTA, MINNEAPOLIS

Pending notice of official award from NSF establishing the Institute, we have begun a search for an Associate Director who will also be appointed to a tenure track Assistant Professorship in the Department of Geology and Geophysics. The 9-month position carries with it half-time responsibility for research and half-time for teaching. The appointee will assist the Director in administration of the Institute. The position will be evaluated annually in accord with departmental and university tenure regulations. The research focus of the Institute will be (a) basic rock magnetism of fine particle oxides, (b) microscopic and macroscopic aspects of interaction between magnetization and stress and (c) separation of stable components of natural remanent magnetization in rocks which have suffered deformation and diagenesis. The applicant should have a Ph.D. in rock magnetism, followed by a minimum of 5 years' experience in self-directed experimental and theoretical research. Salary and benefits will be commensurate with the candidate's record of accomplishments.

Send an application with career documentation, research and teaching interests and names, addresses, and/or fax numbers of 5 persons who can act as references to **Subir K. Banerjee, Department of Geology and Geophysics, University of Minnesota, 310 Pillsbury Drive SE, Minneapolis, MN 55455.** *The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities.* **Deadline for receipt of applications is July 16, 1990**, position available from August 1. Fax: (612) 625-3819, voice phone: (612) 624-5722. E-mail: Geologi@UMNACVX or Geologi@VX.ACS.UMN.EDU.

(NW5394)A

The Division of Medical Genetics, Emory University School of Medicine, is seeking application for the position of **GENETIC COUNSELOR.** Applicants must be eligible for or accredited by the American Board of Medical Genetics, have clinical experience in laboratory-based prenatal genetic counseling and have a Masters Degree in Genetic Counseling. To apply, send curriculum vitae and a synopsis of current and future research interest to:

Dr. Louis J. Elsas, II, Director
Division of Medical Genetics,
Emory University School of Medicine,
2040 Ridgewood Drive,
Atlanta, GA 30322.

Deadline for receipt of application is June 30, 1990.

Emory University School of Medicine is an Equal Opportunity Affirmative Action Employer. (NW5388)A

IMMUNOLOGIST

Applications are invited for a faculty position as an Assistant / Associate Professor in Immunology for the Department of Pathology and Laboratory Medicine at the University of Florida, College of Medicine, with a joint appointment in the Division of Immunology, Department of Pediatrics. This position will be available October 1, 1990 and requires an M.D. and/or Ph.D. degree and post-doctoral research experience. Applicants should have demonstrated ability to conduct high quality research in either molecular or cellular immunology, while candidate will be expected to participate in the teaching of graduate and medical students in the field of Immunology and Immunopathology. Ample research laboratory space is located adjacent to the University of Florida Molecular Biology Core facilities and within the immunobiology unit of the Department of Pediatrics.

Please send CV, letter stating present research efforts and future research goals and the names and addresses of three references September 1, 1990 to:

Richard T. Smith, M.D.
University of Florida
Department of Pathology
Box J 275 JHMHC
Gainesville, Florida 32610

Equal Opportunity Employer/Affirmative Action (NW5360)A

DIVISION OF MEDICAL AND MOLECULAR GENETICS
United Medical and Dental Schools of
Guy's and St. Thomas's Hospitals
Guy's Campus

GRADUATE RESEARCH ASSISTANT

Applications are invited from graduates for a Research Assistant 1B post which is available for three years, supported by the M.R.C. The project involves a search for the gene for Fanconi Anaemia, an inherited disorder of DNA repair, using molecular genetic techniques.

Informal enquiries to Dr. Christopher Mathew on 071-955 4653.

Application by full curriculum vitae giving the names and addresses of three referees to **The Administrator, Paediatric Research Unit, Division of Medical and Molecular Genetics, 8th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT**, by 21st June 1990. Please use the reference F.A. (3721)A

HSC

POSTDOCTORAL POSITION

Available immediately to study the molecular function of the **c-myc oncogene** in negative autoregulation and cellular transformation. **See EMBO J. 9, 1113-1121, 1990.**

Applicants with Ph.D. and molecular biology experience should submit their C.V., & names of 3 references to: **Dr. L.J.Z. Penn, Hospital for Sick Children, Research Institute, 555 University Ave., Toronto, Ontario, Canada M5G 1X8** (NW5384)A

ASSOCIATE/ASSISTANT PROFESSOR

The Department of Molecular Biology and Biochemistry at Wesleyan University invites applications for a position at the Associate tenure or Assistant tenure track Professor level. We seek a person with demonstrated accomplishments in Molecular Biology who uses molecular genetic approaches to study fundamental problems. The successful candidate will be expected to develop a strong research program and to be involved in the Departmental teaching effort.

The position was made possible in part by a grant to the University from Howard Hughes Medical Institue. The Molecular Biology and Biochemistry Department has a strong Ph.D Program, complemented by similar programs in Biology and Chemistry. Areas of strength in the Life Sciences include molecular, cell and developmental biology, genetics, neurosciences, molecular biophysics and biochemistry.

Further information may be obtained from Dr. Lewis Lukens at (203) 347-9411 ext. 2309. Interested applicants should send curriculum vitae, summary of research plans, and names and addresses of three referees to: **Search Committee, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06457.**

Wesleyan University is an Equal Opportunity/Affirmative Action Employer. We encourage applications from minority candidates and women. (NW5354)A

PHYSICAL OCEANOGRAPHER

We intend to hire a tenue-track Assistant Professor in Physical Oceanography to begin in Fall, 1991. Current specialties represented in our program include: general ocean circulation, mesoscale dynamics, bottom boundary layer processes, coastal ocean dynamics, equatorial and coupled ocean-atmosphere dynamics, and chaos in geophysical fluids. The Physical Oceanography faculty traditionally have enjoyed excellent ties with faculty members in Meteorology and Applied Mathematics. A CRAY Y-MP/432 supercomputer is available for use in research.

Applicants, who may be observationalists or theoreticians, should send a letter with their research interests, names and addresses of three referees, and a curriculum vita by December 31, 1990, to **Dr. Y. Hsueh, Chairman, Department of Oceanography, Florida State University, Tallahassee, FL 32306-3048.** Telemail: Y. Hsueh (Omnet). This position is state-funded (academic year).

FSU is an Equal Opportunity Employer. Women and minorities are encouraged to apply. (NW5392)A

Harvard Medical School or Children's Hospital, Boston, MA

Assistant professor or postdoctoral appointments (two)

(1) Enzyme chemist with an interest in molecular biology to work on protein kinases of calcified tissues. (2) Protein chemist. Noncollagenous proteins, protein chemistry and molecular biology. Candidates must have M.D. or Ph.D. and relevant experience. Submit résumés to **Melvin J. Glimcher, M.D., Children's Hospital, Enders Building, 320 Longwood Avenue, Boston, MA 02115.** (NW5383)A

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nature

— the world's most prestigious weekly journal of science

UNIVERSITY OF LEICESTER
Department of Biochemistry
POST-DOCTORAL RESEARCH ASSOCIATE

An MRC-funded position is available in the laboratories of Professor W. J. Brammar and Dr T. M. Harrison for work on the cloning, expression and mutagenesis of sequence encoding human tissue phospholipase A_2. These studies form part of a collaboration with Professor G. C. K. Roberts' laboratory on the structure, mechanism and inhibition of the enzyme, a potential target for anti-inflammatory drugs. The position is for three years from September 1st, at a starting salary in the range RA IA £12,792-14,038 p.a. Applicants should have experience in recombinant DNA work, and experience in the construction of expression systems would be an advantage. Further information can be obtained from Professor Brammar (0533-523441) or Professor Roberts (0533-523054).

Applications, including a full CV and the names of two referees, should be sent to **Prof. W. J. Brammar, Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH.**

Closing date for receipt of applications is 28th June 1990. (3723)A

The Division of Medical Genetics, Emory University School of Medicine, is seeking application for an **ASSOCIATE LABORATORY DIRECTOR** of the **cytogenetics and prenatal diagnosis laboratory.** Applicants should have a doctoral degree and experience in supervising a full-service clinical cytogenetics laboratory, including prenatal diagnosis. To apply, send curriculum vitae and a synopsis of current and future research interest to:

Dr. Louis J. Elsas, II, Director
Division of Medical Genetics,
Emory University School of Medicine,
2040 Ridgewood Drive,
Atlanta, GA 30322.

Deadline for receipt of application is June 30, 1990.

Emory University School of Medicine is an Equal Opportunity Affirmative Action Employer. (NW5387)A

POSTDOCTORAL POSITIONS, NEUROPSYCHOBIOLOGY

Two challenging postdoctoral positions are available to study *in vivo* neurotransmitter mechanisms relating to cocaine reward and craving. Ideal candidates will have extensive experience with *in vivo* voltammetry or *in vivo* brain microdialysis, HPLC, and behavioral methodologies. We seek motivated individuals who can work independently, yet interact well with a large group of scientists studying the neurobiology of drug abuse. Salary is commensurate with NIH guidelines and level of experience. Funding is available for 3 years, subject to satisfactory performance. The positions are available now, but delayed starts are possible for outstanding candidates.

Send curriculum vitae and names of three references to: **Dr. Eliot L. Gardner, Program in Brain and Behavior, Department of Psychiatry, Forchheimer Bldg. G-49, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Ave, New York, NY 10461.**

Affirmative Action/Equal Oppor-

APPTS. WTD

STATISTICIAN AND Pathologist needed? German-American couple. He: BSc. (Zoology), PhD (Biomathematics). Experience in probability theory and statistics. 10 years university experience, 6 years industry (Pharmaceutical): Clinical trials and Preclinical Development. Statistics Packages: SAS, BMDP, SPSS. Languages: Fortran, Pascal, APL, various operating systems. She: MD (German Dr. med.), General Pathology (Ärztin für Pathologie). Experience: Pathology including histopathology (human and laboratory animals). 8 years university experience (pathology, anatomy), 6 years industry experience (Pharmaceutical): Toxicology, toxicopathology. Study director, slide reading, expert reports, discussion with regulatory agencies. Research experience: Developmental pathology (chromosome anomalies), Steroidtoxicology, liver tumors. Languages: German and English. Any location in Europe or North America, but together. Box no. W7388, c/o

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Biopolymer Scientist

The newly established biopolymer group within the Central Research Laboratories of CIBA-GEIGY Ltd. in Basel/Switzerland is seeking a highly motivated and innovative polymer chemist.

Successful candidates will have experience in the chemical and/or biological preparation of biopolymers in the widest sense and like to work in a truly interdisciplinary and competent bio-research team. The skills of our Ph.D. candidate must also include purification and structural identification of those polymers, using modern analytical tools and methodologies.

Our research area will feature mainly polysaccarides including small-scale preparation of layered structures, membranes, mircopaarticles etc. Therefore experience in carbohydrate chemistry, bioorganic chemistry and CAMM is highly desirable. Due to the basic research characteristics of our work, the publication of results is appreciated. We are offering modern employment conditions and excellent research facilities.

Applicants should send curriculum and resume to **Dr. D. Lohmann, c/o Ciba-Geigy Ltd., R-1060.1.14, P.O. Box, CH-4002 Basel/Switzerland.** (W7375)E

CIBA–GEIGY

FELLOWSHIPS

United States Department of Agriculture
Animal and Plant Health
Inspection Service

POSTDOCTORAL FELLOWSHIP PROGRAM

"Apply your special training in modern well equipped U.S. Government and University facilities in conducting critically needed scientific investigations."

Qualifications:
Ph.D.–Biology
Ph.D.–Molecular Entomology/Biology

Application Deadline: August 1, 1990
Salary Range: $24,705 to $42,601 per annum
For Information Call: 1–800–762–2738

U.S. Citizenship Required (NW5369)E

USDA IS AN EQUAL OPPORTUNITY EMPLOYER

POSTDOCTORAL FELLOWSHIP AND POSTGRADUATE SCHOLARSHIP

LINCOLN UNIVERSITY

(Re-advertised)

Applications are invited for a Postdoctoral Fellowship and a Postgraduate Scholarship to study the grazing strategies of beef cattle and other domestic ruminants. This work is part of a continuing program focused on understanding grazing behaviour and its impact on animal and sward performance.

Applicants for the Postdoctoral Fellowship should have completed a Ph.D. degree and have experience with the techniques involved in animal grazing studies. The Fellowship is for 2 years, subject to satisfactory progress. The emolument for this position is at the rate of $NZ35,000 per annum. When making enquiries please quote Vacancy No: 90/32.

Applicants for the Postgraduate Scholarship should have a good degree in animal science, agricultural science, animal ecology or other suitable subjects and be eligible to enrol for a Ph.D. The Scholarship is for 3 years subject to satisfactory progress and is valued at $NZ9,600 per annum. When making enquiries please quote Vacancy No: 90/33.

Further details including conditions of appointment and method of application are available from the undersigned. Applications close 30 June 1990. Please quote the appropriate vacancy number when making enquiries. *Lincoln University is an equal opportunity employer.*

A J Sargison, Registrar
Lincoln University
Canterbury, NEW ZEALAND

(W7391)E

UNIVERSITY OF OXFORD

DEPARTMENT OF EXPERIMENTAL PSYCHOLOGY

RESEARCH ASSISTANTSHIP IN VISUAL NEUROSCIENCE

Applications are invited for a postdoctoral or graduate position to analyse the activity of single neurons in the temporal visual cortical areas of primates. Applications will be particularly welcome from scientists with interests in visual information processing and their underlying neurophysiology. The salary is on the RS1A scale, £10,458-£16,665, or the RS1B scale £9,816-£14,169 (under review).

Applications (including the names of two referees, or enquiries, to **Dr. E. T. Rolls, University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, England**.

The University is an Equal Opportunities Employer.

(3711)P

UNIVERSITY OF YORK

Cancer Research Unit

Department of Biology

POSTDOCTORAL or RESEARCH FELLOWSHIPS

Two fellowships are available for three years in this small but lively Unit and the research is concerned with various aspects of epithelial cell function. The posts would be ideally suited to postdoctoral fellows but high calibre graduates with at least a 2:1 Honours degree might be considered, with the possibility of registration for a DPhil degree.

Post 1 This post, financed by the Imperial Cancer Research Fund, is in collaboration with Dr Jack Cuzick, ICRF. The research concerns a study of DNA repair of ultraviolet light induced DNA lesions in the skin of melanoma patients with a view to establishing if these patients have some repair abnormality. Techniques to be used include the use of monoclonal antibodies in conjunction with 32P postlabelling to study repair rates in skin punch biopsies.
Reference number 8/6307

Post 2 This post, financed by the Health and Safety Executive, concerns studying gene expression in a cultured epithelial cell line. The work will involve an examination of keratin production, stratification and cell number after exposure to a variety of non-genotoxic carcinogens and tumour promoters. Techniques to be used will include Western blot analysis, cell cultures and cell biochemistry.
Reference number 8/6308

For postdoctoral fellows, the salary will be within the range £10,458 to £16,665; for graduates the range will be £9,861 to £12,381. Both scales are currently under review.

Three copies of applications, with full curriculum vitae, naming two referees, and indicating for which post you are applying, should be sent by 21 June 1990 to the **Personnel Office, University of York, Heslington, York YO1 5DD**. Informal enquiries to Dr Colin Garner (0904-432900). (3747)E

UNIVERSITY OF GLASGOW

CRC DEPARTMENT OF MEDICAL ONCOLOGY

Research Assistantship

A vacancy has arisen on a grant funded by the Cancer Research Campaign for a Research Assistant to work on the cellular and molecular biology of resistance to cytotoxic drugs and its modulation. The appointment will run from 1st July, 1990 for 15 months in the first instance. Applicants should have a good honours degree in biochemistry, molecular biology, or a related subject; some experience of tissue culture and/or molecular techniques would be beneficial but not essential.

Salary will be within the RA1B scale, £11,399-£13,495 per annum.

Applications with an up-to-date c.v. and the names of two referees should be sent by 15th June, 1990 to **Dr. R. Ian Freshney, CRC Department of Medical Oncology, Alexander Stone Building, Garscube Estate, Bearsden, Glasgow, G61 1BD,** from whom further information may be obtained (Tel. 041 330 4127).

(3713)P

THE SAINSBURY LABORATORY

JOHN INNES CENTRE FOR PLANT SCIENCE RESEARCH

POST-DOCTORAL FELLOWSHIP

Applications are invited for a three-year post-doctoral fellowship to work on a project on the molecular biology of the fungus *Gaeumannomyces graminis* in relation to Take-all disease of cereals, financed by a grant from the Agricultural and Food Research Council. Applicants should have research experience in molecular biology or a related subject.

The appointment will be made at an appropriate point on the University Research and Analogous Scale 1A (currently £10,458-£16,665).

Contributory Superannuation Scheme. Equal Opportunities Employer.

Further details about the project may be obtained from Dr A. E. Osbourn. Applications quoting reference no SL/481 with CV and names of two academic referees should be submitted by 29 June 1990 to the Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH. Telephone 0603 52571.

(3724)E

POSTDOCTORAL FELLOWSHIP IN PLANT SCIENCE

LINCOLN UNIVERSITY NEW ZEALAND

Applications are invited for a two year Postdoctoral Fellowship in the Plant Science Department funded by the University Grants Committee for research in the area of genetic manipulation/genetic transformation of crop plants. The Department has an established research programme in Cereals to promote gene exchanges between the genomes of different species with the objective of incorporating useful genes into the plant genome of adapted commercial cultivars. Techniques involve both *in vivo* and *in vitro* manipulations. Detection of these exchanges is at the cytological and biochemical levels.

The Plant Science group is expanding the programme to a more molecular approach and is developing techniques of genetic transformation in conjunction with the Department of Biochemistry and Microbiology.

The Postdoctoral Fellow shou[illegible] have a strong plant genetic/cytogenetic background. Experience in biotechnology or molecular biology would be an advantage. Applicants should be familiar with techniques such as electrophoretic separation, DNA extraction, restriction mapping, hybridisation and chromosome banding.

The fellowship is to be taken up before 20 March 1992. Emolument will be at the rate of NZ$35,000 per annum.

Initial enquiries should be made to Dr A.G. Fautrier or Professor R.J. Field, Department of Plant Science (Telephone: 252-811). Further information and conditions of appointment are available from the undersigned which whom applications close on 31 July 1990. Please quote vacancy number 90/23 when making enquiries. Lincoln University is an equal employment opportunity employer.

A.J. Sargison, Registrar, P.O. Box 94, Lincoln University, Canterbury, NEW ZEALAND. Fax: (64)(3) 252-965. (W7392)E

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international
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— EVERY WEEK

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

AUTUMN 1990 AWARDS

Next deadline: 15 August 1990

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1990. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 29 October 1990.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40,69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W7235)E

STUDENTSHIPS

PATERSON INSTITUTE FOR CANCER RESEARCH

Christie Hospital and Holt Radium Institute
Manchester M20 9BX

POSTGRADUATE RESEARCH STUDENTSHIP

Department of Structural Cell Biology

A 3 year Studentship leading to the Degree of Ph.D. is available from 1st October 1990. Renumeration will be compatible with CRC funded institutes. The project to be pursued will involve structural and functional aspects of proliferative and genetic control mechanisms in mammalian nuclei, involving high resolution scanning electron microscopy, biochemical and cell biological techniques, under the supervision of Dr. T. D. Allen, and is designed to dovetail into a larger current programme of research in this area.

Applicants should have, or expect to obtain, a first or upper second class honours degree in an appropriate subject. A detailed C.V. including the names of two academic referees should be sent to **Dr. T. D. Allen, Department of Structural Cell Biology, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX** to arrive no later than 21st June 1990.

AN EQUAL OPPORTUNITIES EMPLOYER. (3770)F

LECTURES

THE EXPERIMENTAL PSYCHOLOGY SOCIETY

announces that the
Eighteenth
Sir Frederic Bartlett Lecture
will be delivered by

Professor R.A. Rescorla

University of
Pennsylvania

'Associative Structures In Instrumental Learning'

6.00 p.m.,
Thursday, 5th July,
1990

DEPARTMENT OF
EXPERIMENTAL
PSYCHOLOGY,
UNIVERSITY OF
OXFORD

South Parks Road,
Oxford

The Lecture will be
open to the Public (3772)K

AFRC INSTITUTE OF FOOD RESEARCH

RESEARCH STUDENTSHIPS

The IFR is the UK's major publically funded centre for independent research on food-related issues. The Institute is currently expanding research at its Laboratories in Reading and Norwich, especially in the areas of Food Safety and Quality, Diet and Health and Biotechnology.

PhD Studentships commencing in October, 1990 are available for the following projects:–

1. Protein Structure Analysis of a Novel Ca2+-binding nodulation protein exported by strains of *Rhizobium Leguminosarum* – Dr P. W. Goodenough and Dr. J. A. Downie, Institute of Plant Science Research, John Innes Laboratory (AFRC funded; – based at Reading Laboratory)
2. Design and NMR analysis of novel protein molecules – (Dr R. W. Pickersgill IFR funded based at Reading Laboratory)
3. Phospholipid binding structures in the cysteine proteinase calpain and its inhibitor calpastatin – Dr C. Crawford and Dr P. W. Goodenough (AFRC/Case award with the Laboratory of Molecular Biophysics, University of Oxford – based at the Laboratory of Molecular Biophysics and Reading Laboratory)
4. Scanning Tunnelling Microscopy of Biopolymers – Dr V. J. Morris (AFRC funded – based at Norwich Laboratory)
5. The role of dietary factors in the maintenance of gastro-intestinal mucosal integrity – Dr I. J. Johnson (IFR funded – based at Norwich Laboratory)

Applications are invited from students who hold or expect to obtain a first or upper second class honours degree in a relevant subject. C.V.'s including the names of two academic referees, should be submitted to:– **The Institute Personnel Officer, Institute of Food Research, Shinfield, READING RG2 9AT.**

Further information may be obtained from the relevant supervisors by telephone:– Reading Laboratory – 0734 883103, Norwich Laboratory – 0603 56122, Laboratory of Molecular Biophysics – 0865 270000. (3759)F

UNIVERSITY OF ABERDEEN

Department of Ophthalmology

RESEARCH STUDENTSHIP

A Medical Research Studentship leading to a Ph.D. degree is available in the Department of Ophthalmology, Medical School, Aberdeen Royal Infirmary, from 1 October 1990.

A major part of the Department's research programme is to investigate factors involved in the growth control of retinal cells involved in the sight-threatening disease of diabetics, named proliferative diabetic retinopathy. We have already identified the presence of a factor in serum from diabetic patients with proliferative retinopathy which stimulates retinal capillary endothelial cell migration. This project is aimed towards identification and characterisation of this factor using biochemical and cell biological techniques.

The supervisors will be Professor John V. Forrester and Dr. Lesley C. McIntosh. The student allowance will be in line with the Research Councils' awards.

Candidates should have a first or upper second class degree in Biochemistry (or Cell Biology or a related discipline).

Further particulars are available from **Dr. McIntosh, Department of Ophthalmology, Medical School, Foresterhill, Aberdeen AB9 2ZD** (tel. 0224-681818 ext. 53783) to whom completed applications with c.v. (2 copies) should be returned by 22 June. (3758)F

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTR

SERC-CASE STUDENTSHIP

Available for 3 years from 1 October, 1990 in collaboration with I.C.I. Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, for work on 'Regulation and disruption of ecdysteroid titres in insects'. A major aim of the project is to examine novel targets in insect moulting hormone (ecdysteroid) biosynthesis – inactivation for exploitation in the development of new insecticides. Informal enquiries may be made on 051-794 4352.

Applicants should possess or expect to have an upper second or first class honours degree in Biochemistry, Chemistry or related subject.

Applications including a full CV and the names of two academic referees should be sent as soon as possible to **Professor H.H. Rees, Department of Biochemistry, The University, PO Box 147, Liverpool L69 3BX**.

An Equal Opportunity Employer. (3730)F

UNIVERSITY OF LEICESTER

Department of Pharmacology and Therapeutics

Ph.D. STUDENTSHIP IN BIOCHEMICAL PHARMACOLOGY

This is an MRC collaborative studentship to work with Dr. M.R. Boarder (Leicester) in collaboration with Dr. S.E. O'Connor (Pharmacology Department, Fisons plc., LOUGHBOROUGH). The student will join a project investigating P_2 purinergic receptors on cultured vascular endothelial cells. The project will characterise the subtypes of P_2 receptors and their effector mechanisms found on endothelial cells derived from different vascular sites.

Applications and enquiries should be directed to: **Dr. M.R. Boarder, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, LEICESTER, LE1 9HN.** Telephone (0533) 522930. (3756)F

The University of Sheffield

RESEARCH STUDENTSHIPS/ASSISTANTSHIPS IN ADVANCED MATERIALS AND MATERIALS PROCESSING are available in the School of Materials at the University of Sheffield. These include:

- ○ Earmarked SERC Studentships on Novel Rapidly Solidified Rare Earth Magnetic Alloys (Prof. H.A. Davies) and Sol-gel Processing of Fibre Reinforced Oxide Matrix Composites (Dr P.F. James).
- ○ Eight SERC Studentships for subjects open to negotiation on metals, ceramics, glasses, polymers and composites and their processing.
- ○ One research assistantship (scale RA/1B) on Development of high lithium aluminium alloys for aerospace applications (Prof. R. Jones).

All the SERC studentships are available for supplementation by collaborating bodies.

Applications are invited from those expecting to graduate with at least a II(ii) Class degree in materials, metallurgy, physics, chemistry, engineering (or related subjects).

For further information on available research topics, telephone 0742-768555, Extension 5467 or write to **Postgraduate Admissions, Departmental Office, School of Materials, University of Sheffield, Mappin Street, Sheffield, S1 3JD.** (3745)F

UNIVERSITY OF READING

School of Plant Sciences

SERC-CASE STUDENTSHIP

Sponsored by the Institute of Horticultural Research, Littlehampton

PLANT MOLECULAR BIOLOGY

A studentship is available in the Department of Botany to work with Dr. C.B. Johnson on the Molecular biology of phytochrome action. The student will spend up to 12 months at Littlehampton, working with Dr. B. Thomas in the Department of Biochemistry and Molecular Biology.

Applicants should hold or expect a first or upper second class honours degree in a relevant subject.

Apply, giving names and addresses of two referees, to **Dr. C.B. Johnson, University of Reading School of Plant Sciences, Whiteknights PO Box 221, Reading RG6 2AS** (Telephone 0734 875123, Ex. 4085) from whom further details can be obtained. (3748)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)

SERC CASE STUDENTSHIP

Studies of Protein Structure and Conformation changes using FTIR and CD Spectroscopy

Applications are invited from candidates who hold or expect to obtain a first class or upper second class Honours degree in an appropriate subject e.g. Biochemistry or Chemistry for a PhD studentship in collaboration with SmithKline Beecham Pharmaceuticals (Dr. D.C. Lee, Welwyn, Herts) to study protein structure using spectroscopic techniques. The project will involve using model systems to extend the available band assignments for the Fourier transform infrared spectra of proteins. Complementary information will be obtained using circular dichroism. These assignments will be used in studying conformational changes in a wide variety of proteins. Techniques for quantitative analysis will be developed. Crystal and solution structures will be compared. Membrane proteins will also be studied with the additional tools of polarised IR and proteolytic digestion.

Applicants should sent a curriculum vitae together with the names and addresses of two referees to **Professor D. Chapman, FRS, Department of Protein and Molecular Biology, RFHSM, Rowland Hill Street, London NW3 2PF.** (3719)F

UNIVERSITY OF NOTTINGHAM

Department of Applied Biochemistry and Food Science
Faculty of Agricultural and Food Sciences
Sutton Bonington, Loughborough, Leics. LE12 5RD

PhD Studentship

Applications are invited for a 3 year AFRC Studentship awarded for the study of the effects of type and quantity of dietary protein on lipoprotein and cholesterol metabolism.

Interested persons who have, or expect to obtain, an upper second or first class degree should send a CV and names of 2 referees to: **Dr Andrew M Salter**. Informal enquiries welcome: (0602) 484848 Extension 8235. (3725)F

POSTGRADUATE RESEARCH STUDENTSHIPS

Two studentships will be available, tenable for three years, at The Natural History Museum commencing in October 1990.

Applications are invited for the following five research topics. The two to receive studentships will be selected following interviews. Arrangements will be made with an appropriate university for joint supervision of the studentships and registration for a higher degree.

Department of Mineralogy

Fluid inclusions and metasomatic processes in carbonatite complexes of eastern Uganda (with Dr A R Woolley).

A study of planetary igneous fragments in meteorites (with Dr R Hutchison).

The mineralogy and surface-geochemistry of platinum group minerals from alluvial and eluvial deposits (with Mr A J Criddle).

Department of Zoology

Systematics, relationships and biogeography of the gastropod genus *Patella* in the North Atlantic and Mediterranean (with Drs J D Taylor, D G Reid and P Rainbow).

The assessment of biodiversity in natural habitats using molecular biology techniques (with Dr D Roberts).

A wide range of scientific facilities is available and students will have full access to the Museum's extensive libraries.

An award of £5000 will be paid, inclusive of London weighting. University fees will be paid in addition.

Applicants must be ordinarily resident in Great Britain, and have a first or second class honours degree; a student graduating in 1990 may be offered an award subject to his/her degree results reaching the required standard.

Application forms, conditions of the awards and further information may be obtained from: Ms S U Elliott,
The Natural History Museum, Cromwell Road,
London SW7 5BD.

Closing date for the receipt of completed forms is 30 June 1990.

The Natural History Museum is an equal opportunity employer. (3741)F

THE
NATURAL
HISTORY
MUSEUM

PRIZES

UNIVERSITY OF NEWCASTLE UPON TYNE

UNIVERSITY OF
NEWCASTLE UPON TYNE

The Robinson Prize

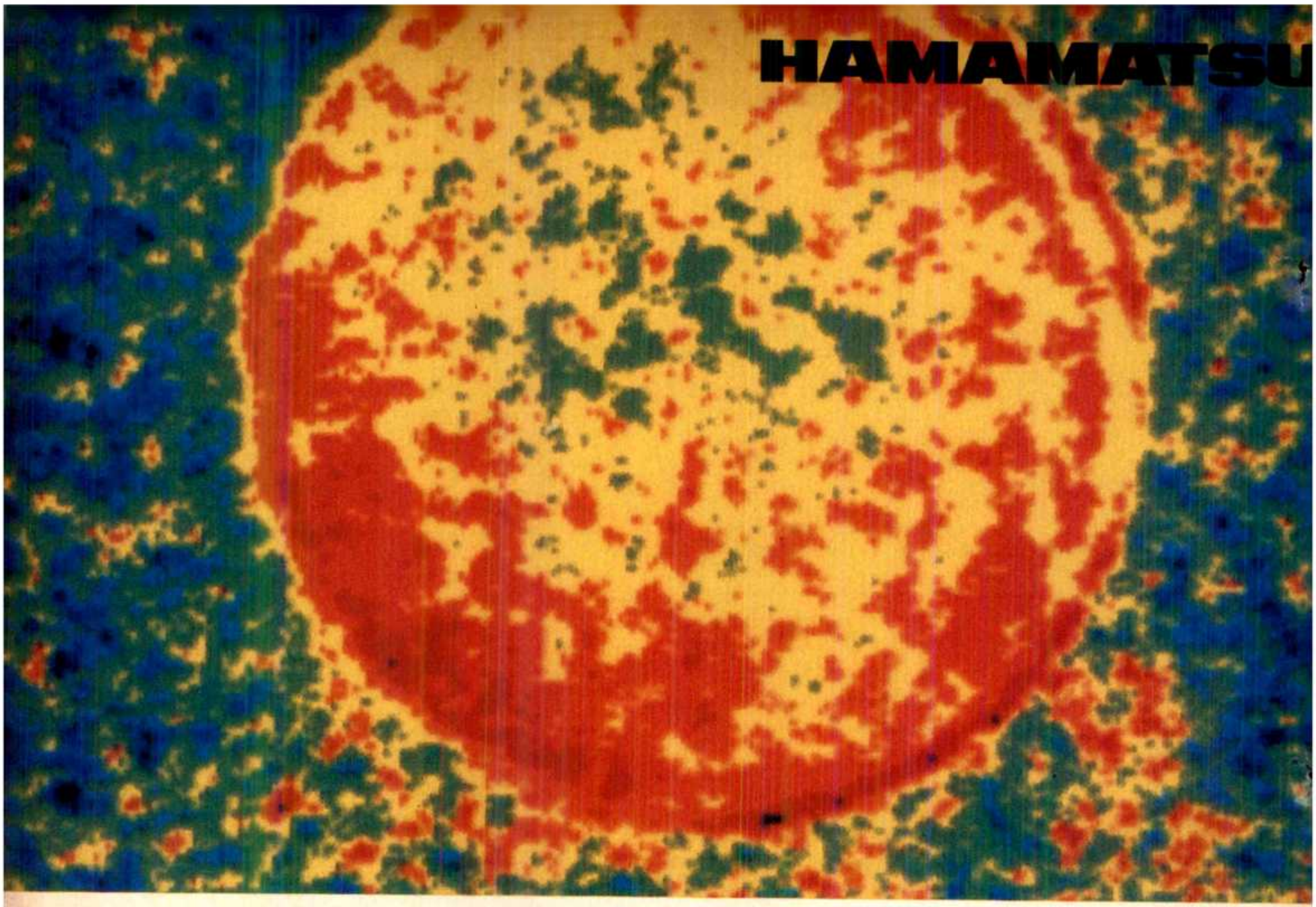
The University of Newcastle upon Tyne, thanks to the generosity of Mr Philip Robinson, MA, wishes to award **The Robinson Prize** for outstanding work in Cosmology. The Prize is £10,000 and will be awarded biennially. The recipient shall be required to deliver a Public Lecture in the University of Newcastle upon Tyne.

The University hopes to award the first Robinson Prize during the 1990-91 academic year, and nominations of distinguished cosmologists from the international scientific community are now invited.

Please write to the **Registar, (Robinson Prize), University of Newcastle upon Tyne,** 6 **Kensington Terrace, Newcastle upon Tyne, NE1 7RU, before 15 July 1990** with nominations which should include a brief account of the candidate's achievement in relation to the purpose of this Prize.

(Further particulars are available from Dr M B Lewis at the above address).

(3742)U



This fluorescence polarity image of a fertilized sea urchin egg obtained with a Hamamatsu ultra-high sensitivity camera shows that the fluidity of the egg's plasma membrane decreases after fertilization. Photonics allows the study of these phenomena without destruction.

LIFE IS A PHENOMENON OF SUBSTANCE.

What makes Life? This is a question no longer to be left only to Philosophy, Theology, or indifference. Scientific research utilizing the principles of Photonics to determine the mechanisms and the reasons for thought, evolution, and for our very existence, will bring us hope, understanding, and Peace.

Teruo Hiruma

Teruo Hiruma
President, Hamamatsu Photonics K.K.

The photon is our business.
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325–6, Sunayama-cho, Hamamatsu City, 430 Japan
Telephone: 0534/52–2141, Fax: 0534/56–7889

THE THIRD INTERNATIONAL CONFERENCE
PEACE THROUGH MIND/BRAIN SCIENCE

August 5th – 10th 1990

Hamamatsu, Japan

Aims of the Conference:

The brain is an organ which, in human beings, governs high-order mental functions such as knowledge, emotion and will. Study of the brain is viewed as forming one of the greatest frontiers upon which science and technology can throw light.

Further elucidation of the brain and mental functions must have a high impact on the advancement of science and technology, and hence open up the way to new developments in science. Moreover, this impetus would play an important role in deepening our understanding of the nature of human-beings, as well as a key role in contributing to world peace.

The aim of this conference is to apply new opto-science and technology to research on the brain and mental functions and thereby further advance this technology in the international world of science.

Presentations

The number of presentations will amount to approximately 30. (Sessions 1–6, Mon-Wed)

Participating countries:

America: The Soviet Union: Sweden: The United Kingdom: Canada: France: Japan: Others.

Attendees

Estimated number to total 200. (50 Non-Japan: 150 Japan)

Language:

English

Executive Committee:

Chairman Teruo Hiruma, President Hamamatsu Photonics K.K.; Kanji Torizuka, President, Fukui Medical School; Ken-ichi Uemura, Professor, Hamamatsu University School of Medicine; Yukio Tateno, Director, Division of Clinical Research, National Institute of Radiological Sciences.

Program Committee:

Chairman Ken-ichi Uemura,	Professor, Hamamatsu University School of Medicine;
Britton Chance	Professor, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania;
Eiichi Tanaka,	Consultant, Hamamatsu Photonics K.K.;
Guy McKhann,	Director of the Mind/Brain Institute, The Johns Hopkins Medical Institutions, Johns Hopkins University;
Henry N. Wagner, Jr,	Director, Divisions of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Johns Hopkins University;
Sydney Brenner	Medical Research Council, Molecular Genetics Unit;
Toshiro Yamasaki,	Section Head, National Institute of Radiological Sciences;
Yasuyoshi Watanabe,	Department Head, Osaka Bioscience Institute;
Yoshiharu Yonekura,	Lecturer, Kyoto University.

SCHEDULE/TIMETABLE

DATE AUGUST	A.M	P.M	EVENING
SUN. 5th		REGISTRATION	REGISTRATION MIXER
MON. 6th	OPENING SPECIAL LECTURES (Dr. Y. YONEKURA)	SESSION 1 BEHAVIOR AND BRAIN (1) Chairman: Dr. G. McKHANN Dr. K. UEMURA	EVENING TALK "WHAT SHOULD WE DO FOR THE FUTURE" Co-ordinator: Dr. H. WAGNER
TUES. 7th	SESSION 2 BEHAVIOR AND BRAIN (2) Chairman: Dr. H. WAGNER, Jr Dr. Y. YONEKURA	SESSION 3 TIME RESOLVED SPECTROSCOPY AND IMAGING OF TISSUES Chairman: Dr. B. CHANCE Dr. M. TAMURA	RECEPTION (18.30-20.30)
WED. 8th	SESSION 4 MOLECULAR ANALYSIS OF NERVE SYSTEM Chairman: Dr. S. BRENNER Dr. Y. WATANABE	SESSION 5 NEW TECHNOLOGY Chairman: Dr. E. TANAKA Dr. S. DERENZO	EVENING TALK "WHAT SHOULD WE DO FOR THE FUTURE" Co-ordinator: Dr. H. WAGNER
THURS. 9th	STATEMENT EXCURSION	SATELLITE SESSION IN JAPANESE EXCURSION	WINE PARTY (10.00-20.30)
FRI. 10th	OPTIONAL FACTORY TOUR	OPTIONAL FACTORY TOUR	

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- Dr. Lewis Thomas, Cornell University Medical College
- Dr. Matthew Scharff, Albert Einstein College of Medicine
- Dr. Irving L. Weissman, Stanford University
- Dr. Max Cooper, University of Alabama at Birmingham
- Dr. Klaus Rajewsky, Institut fur Genetik der Universitat zu Koln
- Dr. Susumu Tonegawa, Massachusetts Institute of Technology
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- Dr. Jonathan Uhr, University of Texas
- Dr. Suzanne Cory, Walter and Eliza Hall Institute, Victoria, Australia
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ROYAL SOCIETY OF CHEMISTRY ANALYTICAL DIVISION BIOLOGICAL METHODS GROUP

1st INTERNATIONAL MEETING ON BIOLOGICAL APPLICATIONS OF SCANNING TUNNELING MICROSCOPY

23rd–24th September, 1990

UNIVERSITY OF NOTTINGHAM

Since the first construction of the Scanning Tunneling Microscope (STM) by Binnig and Rohrer in 1982 the instrument has developed into a major analytical tool for the investigation of single molecules on solid surfaces. In recent years this technique has been applied to reveal structural data at an atomic level on a wide range of biological systems, with great success.

This conference is the first UK based international meeting dedictated to this rapidly expanding area.

Topics to be covered include: Instrumentation, Imaging of Proteins, Peptides, Polysaccharides, DNA, and Organised Lipid Monolayers. The integration of STM image data with computational chemistry and computer graphics will be discussed.

The speakers include Prof M E Welland (Cambridge), V J Morris (AFRC), Prof A Engel (Zurich), Dr Gimzewski (IBM), Prof S M Lindsay (Minnesota), H R Butt (Berlin), D E Jackson (Nottingham).

In addition, STM hardware and software will be exhibited by the manufacturers.

MEETING COSTS

All inclusive cost for food, accommodation and registration:

Members of the Royal Society of Chemistry	£75
Non-members	£85
Full-Time Post-Graduate Students	£40

Registration Details From:

The Biological STM Laboratory
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NOTTINGHAM
NG7 2RD

In view of the likely popularity of this meeting, and the limited places available, early registration is recommended. (3744)C

The 2nd Cold Spring Harbor Laboratory Conference on

CELL and MOLECULAR NEUROBIOLOGY of *APLYSIA*

October 3 - 7, 1990

Organized by:

Hersch Gerschenfeld, *Ecole Normale Superieure*
Leonard Kaczmarek, *Yale University Medical School*
Eric R. Kandel, *Columbia University College of P & S*
Richard Scheller, *Stanford University*
Micha Spira, *Hebrew University*

Topics will include:

- **Single Channels & Channel Modulation**
- **Peptides, Small Molecule Transmitters and Synaptic Transmission**
- **Cell Imaging and Neuronal Growth**
- **Circadian Rhythm**
- **Neural Circuitry and Behavior**
- **Learning and Memory**

The meeting will include formal discussion sessions and poster sessions. The organizers invite submission of abstracts by **July 25, 1990.**

For registration & abstract materials contact:

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Meetings Coordinator
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Cold Spring Harbor, NY 11724
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The course is approved by MRC and limited bursaries will be available to U.K. residents.

Further details of the course may be obtained from **Dr S S Papiha, Course Co-ordinator, Division of Human Genetics, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH — Tel. 091 2226000 ex. 7461.**

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(3720)C

The Northwestern University Cancer Center Presents the First Annual Schweppe Colloquium on

THE MOLECULAR AND CELLULAR BASIS OF DISEASE EPITHELIAL DIFFERENTIATION AND NEOPLASIA

September 30-October 2, 1990
Chicago, Illinois

Keynote Speaker: Stuart Yuspa

SPEAKERS (partial listing):

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Beverley Dale
Elaine Fuchs
Robert Goldman
Elizabeth Hay
W. James Nelson
David Parry
Werner Franke
James Rheinwald
George Rogers
Dennis Roop
Thomas Slaga
Peter Steinert
Tung-Tien Sun
Fiona Watt

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- Molecular structure and dynamics of epithelial cytoskeletons
- Epithelial cell surfaces
- Epithelial-ECM Interactions
- Epithelial neoplasia

Conference Organizers:
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THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

announces

AACR Special Conference in Cancer Research

CHROMOSOMAL AND GROWTH FACTOR ABNORMALITIES IN LEUKEMIA

October 14-18, 1990, Chatham Bars Inn, Chatham (Cape Cod), Massachusetts

SUPPORTED BY A GENEROUS GRANT FROM THE GENERAL MOTORS CANCER RESEARCH FOUNDATION

PROGRAM COMMITTEE Janet D. Rowley, Chicago, IL; Peter C. Nowell, Philadelphia, PA; Donald Metcalf, Melbourne, Australia; Leo Sachs, Rehovot, Israel.

SCIENTIFIC PROGRAM

Sunday Evening, October 14

KEYNOTE ADDRESS George Klein, Stockholm, Sweden

Monday Morning, October 15

REGULATION OF NORMAL AND LEUKEMIC LYMPHOID CELLS William E. Paul, Bethesda, MD; Tadamitsu Kishimoto, Osaka, Japan; Irving L. Weissman, Stanford, CA; Thomas A. Waldmann, Bethesda, MD; Suzanne Cory, Melbourne, Australia.

Monday Afternoon, October 15

GENETIC ANALYSIS OF CHROMOSOMAL ABNORMALITIES IN LYMPHOID NEOPLASMS Stanley Korsmeyer, St. Louis, MO; Timothy McKeithan, Chicago, IL; Michael Cleary, Stanford, CA; Carlo M. Croce, Philadelphia, PA; Terry Rabbitts, Cambridge, England.

Tuesday Morning, October 16

MYELOID GROWTH FACTORS Leo Sachs, Rehovot, Israel; Charles J. Sherr, Memphis, TN; Nicos Nicola, Melbourne, Australia; David W. Golde, Los Angeles, CA.

Tuesday Afternoon, October 16

GROWTH FACTORS IN MYELOID LEUKEMIA Donald Metcalf, Melbourne, Australia; Constance J. Eaves, Vancouver, Canada; James D. Griffin, Boston, MA; Joseph Lotem, Rehovot, Israel.

Wednesday Morning, October 17

GROWTH FACTORS IN MYELOID LEUKEMIA (Continued) Nicholas Gough, Melbourne, Australia; James Ihle, Memphis, TN; Cila Blatt, Rehovot, Israel; Jerry Adams, Melbourne, Australia; Roland Mertelsmann, Mainz, West Germany.

Wednesday Afternoon, October 17

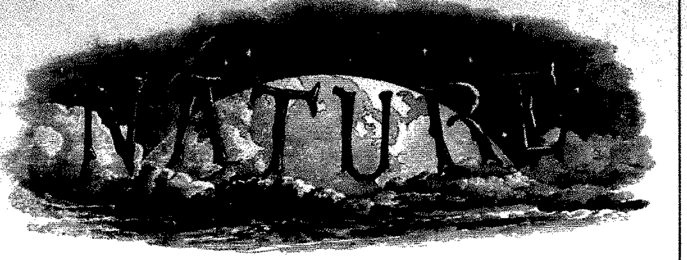
GENETIC ANALYSIS OF CHROMOSOME REARRANGEMENTS IN MYELOID LEUKEMIA Owen Witte, Los Angeles, CA, Gerard Grosveld, Rotterdam, Netherlands; Janet D. Rowley, Chicago, IL; Michelle LeBeau, Chicago, IL.

Thursday Morning, October 18

INTEGRATION AND SUMMARY Peter C. Nowell, Philadelphia, PA; Michael B. Sporn, Bethesda, MD; John Cairns, Boston, MA.

INFORMATION AND APPLICATION FORMS: **American Association for Cancer Research, Public Ledger Building, Suite 816, Sixth and Chestnut Streets, Philadelphia, PA 19106**. 215-440-9300 215-440-9313 (FAX). *APPLICATION DEADLINE*: July 20, 1990. Late applications will be accepted on a space-available basis.

(NW5356)C

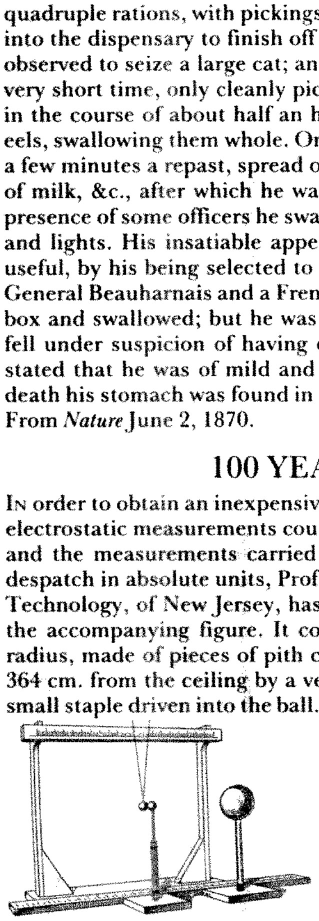


120 YEARS AGO

Perhaps the most extraordinary instance of excessive and depraved appetite on record is that of a French soldier, named Tarare, whose case is described in vol. xxi. of the *Dictionnaire des Sciences Médicales*, by Dr. Percy. He was born near Lyons, and came up to Paris, where his first exploit was to eat a basket of apples — at a friend's expense. On various occasions he swallowed a series of corks and other indigestible materials, which produced such violent colic that he was obliged to attend the Hotel Dieu, and whilst being examined almost managed to swallow the watch-chain and seals of the surgeon in attendance, M. Giraud. Desault, on the occasion of one of these attacks of colic, tried to frighten him out of his gross habits by declaring that it would be necessary to open his stomach, and arranged the instruments; he ran away, and relieved himself by copious draughts of warm water. Soon after he found that his appetite had really increased to an excessive amount, probably owing to the continued irritation produced by these absurd tricks. At 17 years of age, when only weighing 100lb., he could eat 24lb. of beef in as many hours. He now entered the army, and being recognised by the Surgeon-Major, M. Courville of the 9th Regiment of Hussars, he was detained for the sake of curiosity. He was ordered quadruple rations, with pickings and waste meat, but often slipped into the dispensary to finish off a poultice or two. One day he was observed to seize a large cat; and, after sucking its blood, left, in a very short time, only cleanly picked bones, the hair being rejected in the course of about half an hour. He was fond of serpents and eels, swallowing them whole. On another occasion he consumed in a few minutes a repast, spread out for fifteen German workpeople, of milk, &c., after which he was blown out like a balloon. In the presence of some officers he swallowed, at one sitting, 30lb. of liver and lights. His insatiable appetite was for once in his life made useful, by his being selected to convey a correspondence between General Beauharnais and a French colonel, which was inserted in a box and swallowed; but he was caught and soundly thrashed. He fell under suspicion of having eaten a child 14 months old. It is stated that he was of mild and gentle manners and aspect. After death his stomach was found in a very diseased condition.
From *Nature* June 2, 1870.

100 YEARS AGO

In order to obtain an inexpensive apparatus by which the nature of electrostatic measurements could be neatly presented to students, and the measurements carried out before a class with ease and despatch in absolute units, Prof. Mayer, of the Stevens Institute of Technology, of New Jersey, has arranged the apparatus shown in the accompanying figure. It consists of a gilt pith ball of 1 cm. radius, made of pieces of pith cemented together, and suspended 364 cm. from the ceiling by a very fine silk fibre passed through a small staple driven into the ball. The ends of the fibres are attached to the ceiling at a distance of 52 cm. apart, and arranged so that the suspended ball can be raised or lowered, until it is at the same height as a brass ball, also of 1 cm. radius, supported on a glass rod, coated while hot with paraffin wax. A force of 1 dyne acting on the suspended ball deflects it through 13·3 mm., and, as 2° deflection was the maximum employed, the scale was inclined to the horizontal so as to coincide with the chord of an angle of 2°.



If a charge be given to the two small balls when in contact, and when therefore it will divide equally between them, the charge on either in *absolute electrostatic units* equals $D\sqrt{d}/1.33$, where d is the deflection in centimetres of the pendulum from the vertical, and D the distance in centimetres between the centres of the two balls.
"Pendulum electrometer".
From *Nature* May 29, 1890.

50 YEARS AGO

Lord Rayleigh states in *Nature* of April 20 that Dewar's assistant, Lennox, made the first vacuum flask. As one of the few survivors directly concerned with the production of the vacuum flask from its inception, I wish to place on record the facts as I know them.

I first met C. E. Muller, the expert glassblower, in 1887 at 147 Gower Street, where he lived with his brother, Emelius, who was established there as "Artificial Human Eye Maker". At that time I had a laboratory in Charlotte Street, Fitzroy Square, W., for which Muller made the chemical apparatus. At my suggestion Muller began business, in 1887, at 26 Charlotte Street, moving to next door (28) in 1888. In 1891 he removed to 148 High Holborn, the lease of which I helped him to negotiate, where he continued in business until about 1910, with such success that he was able to retire.

When Dewar in 1892 asked Muller to make for him the first double-walled vacuum vessels, they were all made of clear glass, which is sufficient proof, I think, that they were the earliest made. It was then suggested that the internal surface should be silvered, to reduce radiation influx; as Muller had no experience in this, I instructed him in the technique, and actually silvered the first two or three vessels myself.
"History of the Vacuum Flask", George H. Gabb.

Very young setters will instinctively 'point'; Siamese cats are natural retrievers; the wooler rabbit jumps in the air and shaker mice turn somersaults when frightened. C. E. Keeler and H. C. Trimble (*J. Hered.*, 31, 51–54; 1940) describe pedigrees of Dalmatian hounds which indicate that the position under or behind the coach automatically taken by the dog is controlled by heredity. Several interesting examples of instinctive reactions in untrained animals are given. 24 good following dogs and 3 poor followers descended from parents both of which were good coaching dogs. On the other hand, the crosses bad × good and bad × bad produced 7 good, 9 bad and 2 bad dogs respectively. One bitch which always ran with her head between the horses's legs was mated to a dog that trailed behind the carriage. The three pups trailed badly. The same bitch was mated to a dog which ran forward of the front axle. Of their resulting progeny, 6 pups ran in the forward position, and one insisted on running with his nose touching the horse's hind legs.
"Inheritance of Instinct".
From *Nature* June 1, 1940.

10 YEARS AGO

The most profound climatic event of the Tertiary was the terminal Eocene event 34 Myr ago. Botanical data indicate that winters became much more severe, while summer temperatures were little affected. An explanation in terms of a change in the space direction of the Earth's axis is not dynamically acceptable. On the other hand, an ecological disaster of some kind apparently struck the Radiolaria at this time; the latter event is accurately correlated (to a few tens of thousands of years) with the formation of the greatest known tektite strewn field, the so-called North American strewn field, which has recently been shown to extend at least half-way around the Earth. It is suggested here that tektites and microtektites which accompanied this fall, but missed the Earth, organized themselves into a ring system like that of Saturn. The shadow of the rings fell on the winter hemisphere and so produced the observed cooling. The ring lasted between one and several million years.
"The terminal Eocene event: formation of a ring system around the Earth?", John A. O'Keefe.
From *Nature* May 29, 1980.

IN NEXT WEEK'S ISSUE

The most poorly understood of the stellar nucleosynthesis mechanisms — the r process — is the subject of next week's Review Article ■ The Great Attractor meets the cosmic microwave background ■ And in the life sciences, the chemical composition of the orb spider's web is revealed.

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